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6502

Non-specificity of Anuran Hypophyses in Induction of Ovulation in Toads.*

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From the Zoological Laboratory, State University of Iowa.

Experiments were conducted to determine the specificity of the anuran hypophysis in producing ovulation in the toad *(Bufo americanus)*. Houssay, Giusti, and Lascano-Gonzalez[1, 2] advanced the theory of "zoological specificity" when they were unable to secure ovulation in toads *(Bufo arenarium)* using hypophysis implants from several classes of animals, including frogs. They were, however, able to start ovulation in frogs, using hypophysis implants from toads. Adams[3] repeated these experiments, using European species and also made additional experiments using mammalian extracts of the anterior lobe and concluded that only the hormone produced by the toad could stimulate toad ovaries to release their eggs. Recently Bardeen[4] has reported the failure to produce ovulation in *Bufo americanus* after implanting 2 frog hypophyses daily for 15 days.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Emil Witschi.

[1] Houssay, B. A., Giusti, L., Lascano-Gonzalez, J. M., *Compt. Rend. Soc. Biol.*, 1929, **102**, 864.

[2] Houssay, B. A., Giusti, L., Lascano-Gonzalez, J. M., *Rev. Soc. Argent. Biol.*, 1929, **5**, 397.

[3] Adams, A. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 677.

Adams[5, 6, 7] and Burns and Buyse[8, 9] have shown that in urodeles the use of mammalian extracts of hypophysis or the use of heteroplastic implants from various groups of animals causes ovulation in sexually mature females. From the above results it is seen that the toads are the only group of amphibians in which there is a supposed specificity of hypophysis hormone.

In the authors' experiment 2 females of *Bufo americanus* were taken from the general stock on October 14th to be used as recipients of implants of hypophyses from frogs. The implants were placed in the dorsal lymph sac just posterior to the head. Each female received 4 hypophyses daily. The first day each was given 3 implants from *Rana sphenocephala* and 1 from *Rana pipiens* and the following day the donors were all of the latter species. The first female ovulated completely on the third day after having received, altogether, 8 frog hypophyses. The second female likewise ovulated on the third day but after having received 12 frog hypophyses. The eggs were normal, as evidenced by development from natural and artificial fertilization. A male had been placed with each female and the first pair were in amplexus. The males received no implants. As controls a group of 8 females kept for general stock were used. Thus far none of the controls have ovulated. Other experiments were conducted with toads from the same stock, using homoplastic implants in which case 6 of the 8 females used ovulated within 2 to 4 days. Of the 2 not laying eggs, 1 was killed after the fourth day and mature eggs were found in the ovary. The second was found on autopsy to be an immature female.

Our work completes the proof that the hypophysis hormone (anterior lobe) is non-specific in anurans and probably for all amphibia. The possible reason for the failure of other workers to induce ovulation in toads by heteroplastic implants may have been due to physiological conditions of either the recipients or donors, or both.

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[4] Bardeen, H. W., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 864.

[5] Adams, A. E., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 433.

[6] Adams, A. E., *Anat. Rec.*, 1930, **45**, 250.

[7] Adams, A. E., *Anat. Rec.*, 1931, **48**, Suppl. 37.

[8] Buyse, A., and Burns, R. K., Jr., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 80.

[9] Burns, R. K., Jr., and Buyse, A., *Anat. Rec.*, 1931, **51**, 155.

6503

Failure of Hebin to Cause Ovulation in Toads.*

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Hebin, the maturity hormone of pregnancy urine, has been shown by Mazer and Goldstein,[1] and Friedman[2] to produce ovulation in rats, mice, and rabbits. The authors wished to determine if hebin also acts as an inductor of ovulation in toads.

Preparation and Standardization of Hebin. The Wallen-Lawrence and Van Dyke[3] method of preparation of hebin was modified by concentrating the urine under reduced pressure at a temperature below 35°C. and separating the precipitates by filtering instead of centrifuging. In standardization, the equivalent of 0.3 cc. original urine caused the opening of the vagina in each of 3 immature 30-day old female rats; 2 of which showed oestrus cycles and ripe ovarian follicles, but only one of these 2 had an enlarged uterus. This amount was taken as a rat unit. Larger amounts of the preparation showed activity in every case.

Injection of Hebin into toads. Three sexually mature *Bufo americanus* females were placed with males and the former given hebin injections into the dorsal lymph sac. Daily dosages of 1, 2, and 3 rat units, respectively, were used for a period of 5 days. No ovulation occurred during this time. During the next 5 days, injections of 0.5, 1.0, and 1.5 rat units, respectively, were made every 6 hours; making a total of 15, 30, and 45 rat units used. No ovulation occurred during the next 4, 7, and 7 days respectively. The failure to obtain ovulation might be due to one of more possibilities (1) use of immature females, (2) an over-dosage causing degeneration of the ovary, (3) too small of a dosage, and (4) that hebin does not contain the active maturity principle of the anterior lobe of the hypophysis of toads. To test these possibilities, 2 hypophyses from other toads were implanted daily into the above females and ovulation was obtained in all cases on the second day. Since 45 rat units is considered a very high dosage for an animal of this nature,

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Emil Witschi.

[1] Mazer, C., and Goldstein, L., W. B. Saunders Co., Philadelphia, 1932.

[2] Friedman, M. H., *Am. J. Physiol.*, 1932, **99**, 332.

[3] Wallen-Lawrence, Z., and Van Dyke, H. B., *J. Pharm. and Exp. Therap.*, 1931, **43**, 93.

it appears that hebin does not contain the active maturity principle of the anterior lobe of the hypophysis in toads.

Thinking that it might be possible to obtain ovulation in a time of greater sexual activity, both hebin and oestrin were used. Therefore, 4 toads were injected simultaneously with hebin and oestrin into separate lateral lymph sacs with doses of 1, 2, 3, and 4 rat units of hebin and 10, 20, 30, and 40 rat units of oestrin over a period of 4 days; injections being made every 6 hours. No ovulation occurred.

It therefore appears that neither hebin alone nor hebin with oestrin is capable of inducing ovulation in toads. Reichert and his associates,[4] Evans and his coworkers,[5] and Iimuro and Murata[6] have suggested that hebin is not the hormone identical with the gonad stimulating hormone of the hypophysis. Our work with toads substantiates this view.

6504

A Simple Method for Detecting Abnormal Hearts.

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From the Department of Public Health and the Department of Physiology, State University of Iowa.

On the basis of a pulse-ratio method Tuttle and Wells[1] showed quite conclusively that in the case of the normal heart the increase in rate caused by exercise is directly proportional to the intensity of the exercise and that the form of the curve representing the relationship between heart rate and exercise intensity is rectilinear. It occurred to us that if abnormal hearts did not conform to this principle a simple method for detecting them is provided.

By using the pulse-ratio technique data were collected from 32 University freshmen reported as having abnormal hearts. The physicians' report of these cases at the time of examination was as follows: 6 cases of functional murmur, 16 cases of organic lesion in which there was definite evidence of decomposition, 6 cases of organic lesion with no signs of heart failure, 2 cases of neurogenic lesion* and 2 cases which could not be agreed upon as to class. Each case was examined upon 2 different occasions by the pulse-ratio test.

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[4] Reichert, F. L., Pencharz, R. I., Simpson, M. E., Meyer, K., and Evans, H. M., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 843.

[5] Evans, H. M., Meyer, K., and Simpson, M. E., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 845.

[6] Iimuro, Syozo and Murata, Miyakita., *Trans. Japan. Path. Soc.*, 1931, **21**, 156. (From *Chem. Abst.*)

[1] Tuttle, W. W., and Wells, Geo., *Arbietsphysiol.*, 1931, **4**, 519.

The data show that in cases of functional murmurs, compensated organic lesions and neurogenic lesions, the response to exercise is exactly the same as with normal hearts. However, where non-compensated organic lesions are present the response to exercise is not always directly proportional to the intensity of the exercise and the curve representing the response is never rectilinear.

In 2 cases in the group there was a difference of opinion as to the nature of the heart lesion. Repeated examination by the pulse-ratio technique placed both of these cases in the non-compensated organic lesion group. In these cases the physician agreed that there was sufficient evidence of heart lesion to justify restricted activity.

The results of this investigation indicate that the pulse-ratio technique is reliable in detecting non-compensated organic lesions. The agreement between the results of this test and the other methods of diagnosis indicates that this simple procedure is of value as a guide in determining what restriction, if any, should be placed on individual activity. Furthermore, it has proven of use as one of the indices of the condition of the heart in the physical examination.

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* Hearts which are very easily accelerated by reflex stimulation.

Peiping Section.

Peiping Union Medical College, November 10, 1932.

6505

Experimental Osteochondritis of the Femoral Head.

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The etiology of osteochondritis of the head of the femur has never been proved clinically or experimentally. The theories may be grouped under the headings of infection (Kidner, McWhorter, Phemister), circulatory disturbances (Axhausen, Leriche and Policard, Bentzon, Zeimansky and Lipmann), and mal-development (Calot and Mürk Jansen). The second of the theories seemed the most likely, consequently it was tested by experiments in dogs and rabbits in which the circulation of the head of the femur was interfered with, and the bony changes were noted from time to time, to see if they might similate those of the human disease known as osteochondritis (Legg-Calvé-Perthe's disease).

Seven dogs from 2 to 4 months old and 16 rabbits from 2 to 3 months old were divided into 4 groups. They were operated upon aseptically and 4 different degrees of interruption of the vascular supply of one femoral head instituted. Roentgenograms were made shortly after the operation and at the time of the autopsy. Autopsies were performed at periods varying from 2 weeks to 4½ months after operation. Cultures were taken from the hip joints operated upon, and the upper ends of both femurs were removed for gross and microscopic study. Group I. Two dogs and 4 rabbits. The round ligament of one hip joint was severed. All of the animals treated with this method developed dislocation of the operated hip within one month and, therefore, were discarded. Those treated by the other methods did not present this complication. Group II. Three dogs. Alcohol was injected into the periosteum of the neck of the femur. Gross and microscopic examination of the specimens showed that the bone and cartilage of the femoral head remained normal. Group III. Four rabbits. Alcohol was injected into the periosteum of the femoral neck; the periosteum was stripped from the entire neck of the femur. Microscopic sections showed nothing of importance except for a few small areas of aseptic necrosis of the bone. Apparently, the circulatory embarrassment caused only a limited necrosis, reparable without gross changes in the head. Group IV. Eleven rabbits. The round ligament ligated with a silk suture; the periosteum stripped from the entire femoral neck; silk suture placed around neck. In one dog (10 weeks) the femoral head appeared normal grossly but in all others it was greatly roughened and flattened. Microscopically in all cases, the bone and cartilage of the head were uniformly and markedly atrophied and, in many areas, necrotic. Of particular interest were well defined cystic spaces which represented areas of degeneration of the medullary bone and of the marrow tissues. As early as 2 weeks after operation, new vessels were seen growing from the shaft along the surface of the neck and the necrotic areas were found to be undergoing repair.

It is concluded that the circulatory deficiencies of the head of the femur produced in Group IV caused gross and microscopic changes similar essentially to those of the human lesions of osteochondritis. It seems probable that the dog in Group IV without flattening of the femoral head would have developed that deformity if it had been kept alive a little longer, since the collapse in the other cases was very probably due to weakening of the internal structure and weight-bearing.

6506

Acidosis in Cholera. I. Path of Displacement of Serum Acid Base Equilibrium.

S. H. LIU, S. H. WANG AND C. FAN.

From the Department of Medicine, Peiping Union Medical College.

During the outbreak of cholera in the summer of 1932 we had the opportunity of studying 28 cases from the standpoint of their serum acid-base equilibrium. With the micro-acid-base method of Shock and Hastings,[1] cell volume, pH, and total CO_2 of blood were determined and from these values serum bicarbonate content in millimols per liter and carbon dioxide tension in millimeters of mercury were calculated. These observations were made on admission before treatment and repeated thereafter almost daily throughout their hospital sojourn. Altogether 222 observations were made.

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[1] Shock, N. W., and Hastings, A. B., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 780.

During the acute stage of cholera there was invariably acidosis. Serum pH obtained from this series of cases on admission averaged 7.28, bicarbonate content 14.1, and CO_2 tension 30.7. The lowest pH recorded in this series was 7.07, the lowest bicarbonate content 8.2, and the lowest carbon dioxide tension 16.6, as compared with the normal average values of 7.40, 26.6, and 44.9, respectively.

With recovery following intravenous therapy with either saline or alkali or both, pH returned to normal fairly rapidly and was followed by the return of bicarbonate. Soon after these values returned to normal they were often shifted to the alkaline side. Fourteen cases in this series which were followed for a relatively long period during their convalescence showed a tendency to alkalosis, irrespective of whether or not they had received alkali. During the stage of alkalosis these cases showed on the average pH 7.51, bicarbonate content 31.3 and CO_2 tension 42.3.

In the 4 cases of death in this series the intravenous treatment was capable of bringing the serum pH and bicarbonate content to normal, but this was only temporary.

When all the observations on the 28 cases are grouped according to their pH ranges, and the average values of pH, bicarbonate and CO_2 tension of each group are plotted on triaxial coordinate paper, the general trend of displacement of serum acid-base balance may be described as follows: A change in bicarbonate involves a change in both pH and CO_2 tension. Similar to experimental displacements of acid-base balance by the administration of acids or alkali,[2] the ratio of change in bicarbonate :pH :CO_2 tension is approximately 3:2:1, when bicarbonate and CO_2 tension are also expressed logarithmically.

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[2] Liu, S. H., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 781.

6507

Acidosis in Cholera. II. Changes in Serum Electrolytes.

S. H. LIU, C. FAN AND S. H. WANG.

From the Department of Medicine, Peiping Union Medical College.

Of the series of cases of cholera reported in the preceding paper,[1] more detailed studies were made on 13 in order to gain a more complete picture of the changes in serum electrolytes. Besides pH and bicarbonate content, we also determined serum total base, chloride, phosphate, proteins and lactate. These determinations were made during the acute stage, usually before treatment, and repeated at intervals during the course in the hospital. Altogether 48 determinations were made on the 13 cases.

TABLE I.

Showing average concentrations in serum electrolytes during acute stage and during convalescence. Values except pH are expressed in milli-equivalents per liter.

	Acute Stage (1)	Convalescence* (2)	Difference (1)-(2)
pH	7.27	7.45	0.18
Total base	138.4	152.5	—14.1
HCO_3	14.8	30.7	—15.9
Cl	92.2	99.2	— 7.0
HPO_4	4.0	1.8	2.2
Proteins	15.8	13.5	2.3
Lactate	6.8	2.3	4.5
Undetermined	4.8	5.0	— 0.2
Sum of anions	138.4	152.5	—14.1

* Figures in this column represent averages of 12 cases, as one patient died during the acute stage.

The average values obtained during acute stage on admission and those on recovery are presented in Table I. It is noted that in the acute stage besides the decrease in pH and bicarbonate content, there is a distinct reduction in serum total base and chloride concentrations and an elevation of phosphate, lactate, and, to a lesser extent, proteins. These changes tend to disappear with recovery from acute symptoms, although the rate of return to normal of individual constituents may vary considerably. When the values obtained on admission are compared with those on discharge, it may be observed that during the acute stage sufficient base is lost from the body to lower the serum base concentration to the extent of 14.1

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[1] Liu, S. H., Wang, S. H., and Fan, C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 417.

milli-equivalents per liter. Added to this alkali deficit from loss of base is the increased demand for base from the increase of 2.2 milli-equivalents in phosphate, 2.3 milli-equivalents in protein and 4.5 milli-equivalents in lactate, totalling 9.0 milli-equivalents. The combined alkali deficit from loss of base and from increased demand for it amounts to 23.1 milli-equivalents. This alkali deficit is shared by bicarbonate and chloride. Chloride, being a fixed acid, is decreased only 7.0 milli-equivalents, leaving the greater part of the burden to bicarbonate, which is decreased 15.9 milli-equivalents, giving as a result marked acidosis.

6508

Nature and Result of Animal Tissue Reactions to Cellulose.

J. W. SPIES, F. D. MANDEVILLE AND F. J. AWDZIEWICZ.
(Introduced by C. M. Van Allen.)

From the Department of Surgery, Yale University School of Medicine, New Haven, Conn.

Experimental evidence of the biological decomposition of cellulose was first presented 80 years ago,[1] and many articles have since been written concerning this phenomenon. The amassed knowledge has been well summarized.[2, 3] We will therefore stress a few salient points.

The bacteria and fungi that live on cellulose exist for the greater part in soil, dung, or ensilage. Several saprophytic bacteria, as *B. cereus* and *B. mesentericus* have been reported as acting on cellulose,[4] but this has not been substantiated.[5] At first it was thought that symbiosis was necessary for the dissolution of cellulose, but further work demonstrated the ability of pure strains to perform this task. The action in any case is the result of a cellulase, and this has been repeatedly isolated.[6, 2, 3] It has been claimed that cellulase has only a slight effect on untreated cellulose but reacts vigorously with that which has been subjected to the action of some chemical, especially a copper ammonium salt.[7]

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[1] Mitcherlich, (Quoted in Bradley & Rettger and in Kellerman and McBeth, references Nos. 2 and 3).

[2] Bradley, L. A., and Rettger, L. F., *J. Bact.*, 1927, **13**, 321.

[3] Kellerman, K. F., and McBeth, I. G., *Centralbl. f. Bakt.*, 1912, **34**, 485.

[4] Thaysen, A. C., and Bunker, H. J., *Biochem. J.*, 1926, **20**, 692.

[5] Spies, J. W., Mandeville, F. D., and Awdziewicz, F. J., unpublished studies.

[6] Belehrádek, Jan, *Arch. Int. Physiol.*, 1922, **17**, 260.

The invertebrates[8] which digest cellulose are those capable of utilizing it as a food, such as certain Sarcodina, Mollusca, and Flagellata. No enzyme has been found in the vertebrates that will digest cellulose, but the process is apparently accomplished with the aid of intestinal bacteria. The opinion is commonly held that tissue responses to trauma, regardless of the presence or absence of infection, are unable to affect the integrity of cellulose.

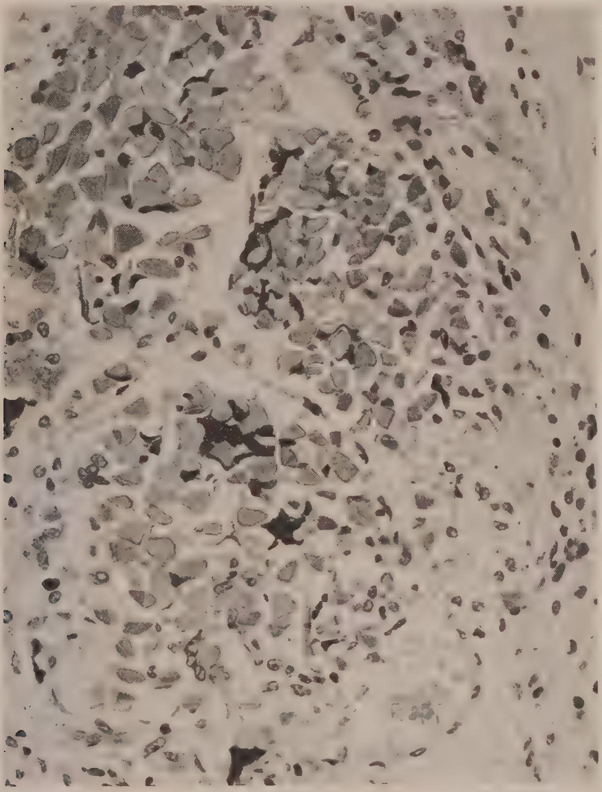

FIG. 1.

Mag. = x-295. Cotton imbedded subcutaneously for 50 days. This illustrates an early phase in the absorption of cellulose. There is some giant cell reaction, but the cotton remains intact. The swelling and hyalinization of the fibers has not begun.

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[7] Karrer, P. u. Illing, H., *Kolloid-Z.*, 1925, **36**, (Spec. No.) 91.

[8] Trotman, S. R., and Sutton, R. W., *J. Soc. Chem. Indus.*, 1924, **43**, 190T.

Cellulose of cotton, linen, or artificial silk sutures has always been considered insoluble in the human tissues.[9] Such material has been found at the bottom of intestinal ulcers following operations.[10, 11, 12] Unimpaired linen sutures have been reported 120 days following their placement in dogs. Intact gauze sponges have been recovered years after insertion in patients. Artificial silk sutures have been found unabsorbed in infected wounds weeks after introduction. It has been reported that artificial silk sutures are not absorbed by the tissues of the guinea pig and that little tissue reaction follows the incorporation of this material.[13] It was, therefore, considered that this artificial silk was an excellent suture material for plastic operations.[14]

Our interest in this problem arose from accidental findings in experiments[7] in which cotton pledgets enclosing tubercle bacilli disappeared from the subcutaneous tissues of guinea pigs and rabbits without leaving any discoverable trace as to the mechanism by which the disappearance was effected.

A cotton pellet and a strand of cotton were sutured loosely to the

TABLE I.

Cotton Imbedded in and Removed from the Tissues of Guinea Pigs.

Animal No.	Days in Peritoneal Cavity	Days in Muscles of Back	Days in Muscles and Subcutaneous Tissues of Thigh	Wound Infection
1	24	24		Abdominal Wall*
2	111		50	”
3		86	50	”
4	96		50	”
5		88	50	”
6		58	50	Back
7		8		”
8	58		50	None
9		111	50	”
10		3		”
11	86		50	”
12	1			”
13	58		50	”
14	21			”
15	58		50	”

* No infection of peritoneal cavity.

In compiling the above data, only those guinea pigs were used from which the cotton could be recovered, thus eliminating 9 of the animals.

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[9] Keen, W. W., *Surgery*, W. B. Saunders, Philadelphia and London, 1914, **5**, 601.

[10] Mall, F., *Johns Hopkins Hosp. Rep.*, 1896, **1**, 76.

[11] Mitchell, A. B., *Ann. Surg.* 1911, **54**, 806.

[12] Soresi, A. L., *Ann. Surg.*, 1915, **61**, 328.

[13] Durck, H., *Deutsche Z. f. Chir.*, 1924, **189**, 31.

[14] Roith, O., *Deutsche Z. f. Chir.*, 1924, **189**, 46.

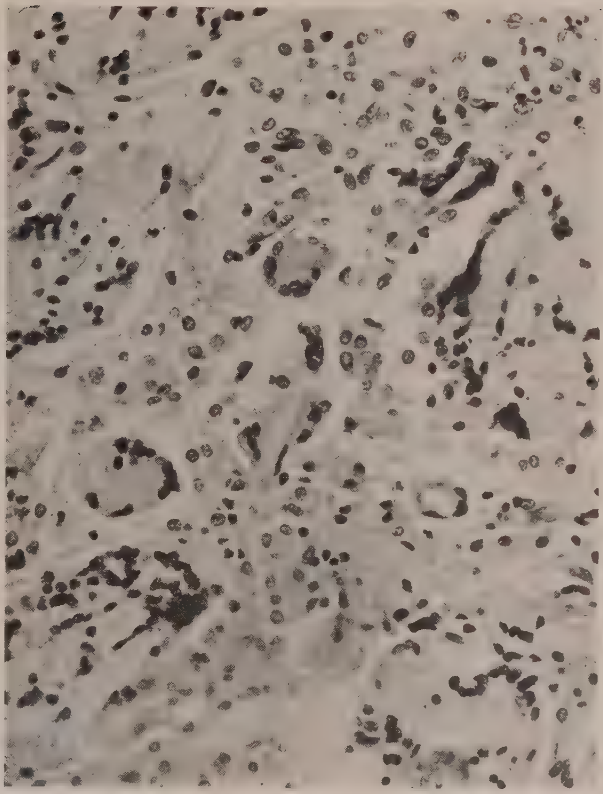

FIG. 2.

Mag. = x-295. Cotton imbedded intraperitoneally for 111 days. Giant cells have engulfed and partially destroyed the cellulose. In some areas the cotton fibers are distinct; in others they have disintegrated through swelling and hyalinization.

inner surface of the parietal peritoneum in 8 guinea pigs. Cotton strands and pellets were inserted into the muscles of the back in 6 other animals, and placed in the peritoneal cavity in one animal. Nine guinea pigs were inoculated subcutaneously in the thigh with a cotton pellet. The cotton pellets were enclosed in a single layer of gauze, the open end of which was tied across with black silk; and the strands of cotton were in the form of loose bundles, the ends of which were also tied across with a single black silk thread. Scrupulous aseptic technic was employed throughout the procedures.

Until the wound was healed, it was necessary to isolate each animal, because cage mates would almost always rapidly bite out

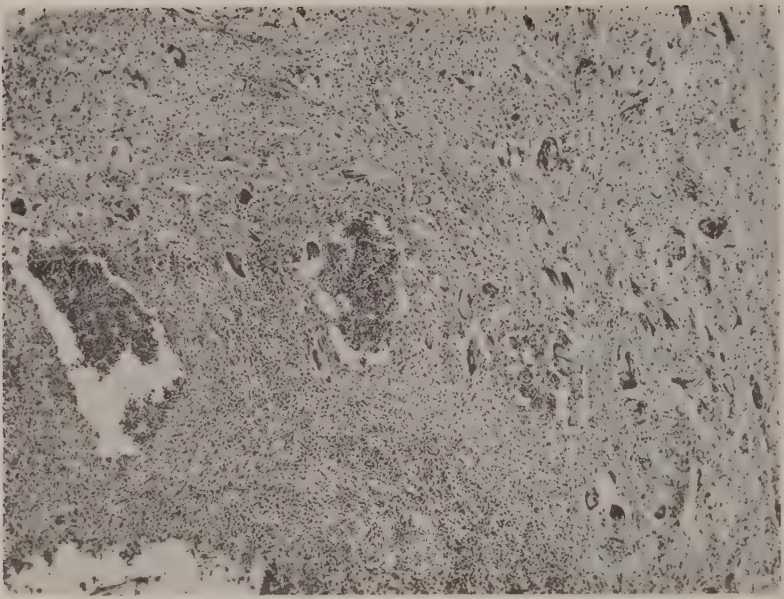

FIG. 3.

Mag. = x-68. Cotton imbedded intraperitoneally for 86 days. In the lower left hand portion giant cells are absent, and the tissues are infiltrated with polymorphonuclear leucocytes and unidentified round cells. This demonstrates that the cellulose has disintegrated in the infiltrated area.

each other's sutures. Before isolation was rigidly enforced, some of the abdominal wounds were bitten open, in several instances requiring debridement with repair of the fascia and skin. Apparently the peritoneum was never injured. The wounds of the back were more satisfactory, only one being seriously infected. None of the wounds of the thigh suppurated.

The pellets were recovered at the end of one to 111 days. In every case, when the pellet had been imbedded 50 or more days, there was encapsulation of the mass with fibrous tissue, varying from being barely discernible to 2 mm. in thickness.

Summary. Cotton pellets and strands were placed intraperitoneally, subcutaneously, and intramuscularly in 24 guinea pigs and were recovered grossly intact in 15 animals after one to 111 days. In the remaining 9 guinea pigs it was thought that the cotton was removed from the wound and eaten by the animals, since no trace of it was found either before or after death.

Microscopic examination of the removed cotton and adjacent tissue demonstrated a moderate phagocytosis of the cellulose by giant cells and a dissolution of same in the areas of inflammation. A marked hyalinization of cotton fibers was frequently noted, and this was interpreted as denoting partial disintegration. The destruction of the cellulose was greater in the peritoneal cavity than in the muscles or subcutaneous tissue. Studies are now in progress to determine the absorbability of natural silk before and after treatment with various substances.

6509

Reconstruction of the Ureter by Means of Bladder Flaps.

J. W. SPIES, C. E. JOHNSON AND C. S. WILSON.
(Introduced by C. M. Van Allen.)

From the Department of Surgery, Yale University School of Medicine.

After operations on the lower one-third of the ureter, strictures usually form despite the most careful efforts to prevent them. Considerable clinical and experimental data indicate the difficulties of securing a satisfactory ureterovesical anastomosis and describe methods which it was hoped would solve the problem.

It seemed to us that a modification of the Janeway gastrostomy would offer a procedure for replacing the resected lower portion of the ureter with a tube constructed from the bladder. The literature revealed that Boari conceived a similar idea but his observations were limited to one dog which lived in apparent good health for 4 years after the operation but was never autopsied.[1]

Various procedures were tried, each consisting essentially in turning a flap of bladder wall and constructing a tube of it into which the ureter was placed. The most satisfactory type for the preservation of blood supply to the bladder tube or pouch was to turn a flap from the fundus downward, having its broadest portion attached to the base of the bladder and preferably at a point entered by large blood vessels. Likewise, it was desirable to have the base of the flap slightly broader than the free end.

In dogs, this procedure afforded an easy transplantation of the ureter even after a resection of about 5 cm. of the lower part. If a greater length of ureter were removed, the flap could be turned from the base upward towards the fundus as advocated by Boari. In this instance, however, the blood supply to the flap was less abundant, and the position of the artificial tube or pouch varied with the size of the bladder.

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[1] Boari, A., *Chirurgia dell' uretere. Con prefazione del Dott I. Albaran,* 1900, **14**, 444; *Contributo sperimentale alla plastica dell uretere, Atti della Accad. delle scienze mod. e naturali di Ferrara. Seduta* 27 *maggio* 1894.

Although one could easily maintain the integrity of the bladder tube and could easily effect an anastomosis, a stricture almost invariably formed at the junction of the ureter and the bladder, which in itself defeated the purpose of the operation. Therefore, studies were directed toward finding a suitable type of anastomosis between the normal bladder and normal ureter, and this procedure[2] was applied satisfactorily to uniting the ureter with the tube constructed from the bladder.

6510

Ureterovesical Anastomosis: An Experimental Study.

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(Introduced by C. M. Van Allen.)

From the Department of Surgery, Yale University School of Medicine.

Due to the formation of strictures in the ureter when transplanted into a tube constructed from the bladder,[1] studies were directed toward finding a satisfactory method of anastomosing the normal ureter with the normal bladder. Several modifications of the Coffey type of implanting the ureter into the sigmoid were employed.[2]

Our most satisfactory anastomosis was obtained by the following technic. The ureter was cut across near the bladder between 2 silk ligatures. The bladder was opened anteriorly. The proximal portion of the ureter was led through the posterior bladder wall at about the same level and slightly medial to the normal entrance of the incised ureter. This was easily done by using a large curved, non-cutting needle which carried through the loose ends of the ligature previously tied around the proximal end of the cut ureter and which then could be used to pull the ureter into the bladder cavity for the desired distance (usually about 1.5 cm.). To accomplish this no incision or dilatation with instruments was needed. The mucous membrane of the bladder was incised downward for a distance of about 1 cm. from the hole made by the artificial entrance of the ureter. The corresponding portion of the ureter within the bladder was next placed in the defect created by the incision of the mucous membrane which was then sutured over the ureter. This left about 1 cm. of the ureter lying between the muscle and the mucous membrane. Subsequently the ureter projecting into the bladder cavity was cut squarely across just proximal to the silk ligature, and usually the end was split into 2 equal parts, each of which was turned back and sutured to the mucosa of the bladder. In some instances, a portion of the end was incised in an oblique manner and the most distal point was sutured to the bladder mucosa. Catgut sutures were employed inside the bladder as non-absorbable sutures caused the collection of urinary salts.

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[2] Spies, J. W., Vermooten, V. D., and Wilson, C. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 426.

[1] Spies, J. W., Johnson, C. E., and Wilson, C. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 425.

[2] Coffey, R. C., *Brit. J. Urol.*, 1931, **3**, 353.

The operated dogs were observed for varying periods of time. Urograms, autopsies, and histological studies were made.

6511

Postoperative Behavior of the Diaphragm and Ribs in Dogs.

C. M. VAN ALLEN AND T. S. JUNG.

From the Departments of Surgery and Radiology, Peiping Union Medical College.

For a few days after upper abdominal operations in man the thoracic parietes are characteristically altered. Both the diaphragm and the ribs are markedly elevated, so that the space occupied by the lungs is shorter and broader; also, the diaphragmatic and costal movements are much reduced. The cause of this has been assigned to several factors: tight surgical dressings about the abdomen, reflex spasm of the abdominal and intercostal muscles, reflex inhibition of the diaphragm, gastro-intestinal distention, and pneumoperitoneum. Clinical studies[1, 2, 3] have furnished very suggestive evidence, but opinions are still conflicting as to the chief cause. We felt that, if laboratory animals also present these postoperative changes, they would be better adapted than man to the study of the

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[1] Muller, G., Overholt, R., and Pendergrass, E., *Arch. Surg.*, 1929, **19**, 1322.

[2] Potey, D., *Brit. J. Surg.*, 1930, **17**, 487.

[3] Jung, T., and Van Allen, C., ready for publication.

causes and principles involved. Therefore, standard upper abdominal operations were performed on a series of dogs, with the essential surgical circumstances the same as those used in man (except, of course, the confinement in bed), and the diaphragm and ribs were examined. Other series of dogs were used as controls.

Five dogs (Group I) were employed as follows: All were fed as usual on the morning of the first day. Six hours later, morphine and atropine were given by hypodermic, and 1 hour after that a perfectly symmetrical anteroposterior roentgenogram of the chest was made, the x-rays being centered over a point 10 cm. cephalad to the xiphoid, by a specially devised method. The exposure was made at the expiratory phase of quiet breathing (as the most comparable phase in these animals whose respiration cannot always be regularized as to the depth of inspiration). Fluoroscopy was then done to determine the range of motion of the diaphragm and ribs with deepest breathing, produced by putting on a mask and cutting down the airway to a point barely compatible with the ability of the dog to maintain adequate pulmonary ventilation. One hour later the animal was etherized and operated upon with aseptic surgical technique. A long right rectus incision was made and one of 3 visceral operations was done: gastrotomy (2 dogs), partial gastrectomy (2 dogs), and cholecystectomy (1 dog). The incision was then sutured, *without* care to avoid leaving air in the peritoneal cavity, and a firm bandage was applied to the abdomen. Twenty-four hours after the operation a small dose of morphine was given, and 1 hour later the bandage was removed and the roentgenographic examination was repeated. Food was withheld until after that.

A second group of 5 dogs received exactly the same treatment except that the visceral trauma was omitted. The wound was as long, was traumatized as much, and was kept open as long, as in the first group. A third set received the same treatment, with the omission of the visceral trauma and the incision; and a fourth had the same treatment, with the omission of the visceral trauma, incision and etherization.

The pre- and postoperative x-ray films of each animal were compared as follows: A point was placed at the center of the upper margin of the body of the first thoracic vertebra, another in the mid-line on the profile of the diaphragm, and two more on the profile of the diaphragm on both sides of, and 4 cm. away from, the second point. The distance between the first point and each of the 3 others was measured, and the average was taken, to represent the position of the diaphragm in that roentgenogram. The value ob-

TABLE I.

Changes in position of the diaphragm and ribs after the various procedures.

Group	Procedure	Av. rise of diaphragm	Av. narrowing of chest
		mm.	mm.
I	Visceral operation	1	17
II	Incision	0	7
III	Anesthesia	—1	6
IV	Morphine	—1	1

tained from the postoperative film was subtracted from that obtained from the preoperative film, and this figure (plus or minus) was used to represent the amount of elevation of the diaphragm after operation. Finally, the greatest distance between the inner surfaces of the seventh ribs, as shown in the film, was measured; and the postoperative value was subtracted from the preoperative value, to obtain a figure which would represent the amount of narrowing of the lower part of the chest after operation.

In none of the dogs did the diaphragm show distinct or consistent alterations after the experimental procedures. It was a little depressed in some instances and a little elevated in others, so that the average for each group was extremely close to zero (Table I). However, the spread of the ribs was consistently and markedly reduced after the operation in the dogs of Group I, consistently (almost) and slightly reduced in those of Groups II and III, and without consistent or marked change in those of Group IV; so that the average of the first group was 1.7 cm., that for the second and third groups was 0.7 and 0.6 cm. respectively, and that for the fourth group was 0.1 cm. The fluoroscopic results could not be reduced to figures so precisely, but they showed no appreciable change in the ranges of motion of either the ribs or the diaphragm after operation. Since the diaphragm was unaltered as to either the expiratory position or the maximal range of motion, it was concluded that the inspiratory position also was unaltered.

Therefore, the dogs showed only one consistent respiratory abnormality after operation, *i. e.,* narrowing of the chest, which is apparently caused partly by the visceral trauma and partly by the anesthesia. The abdominal incision evidently played no part, since the third group showed just as much narrowing as the second. The removal of the abdominal bandage before making the postoperative examinations was not responsible for the negative results, for we have found from other experiments[4] that the bandage has to be applied very much more tightly than is done ordinarily to retain dressings, in order to affect the action or position of the diaphragm. Since the abnormality seen in these dogs is quite different from those in man, we shall not study its mechanism further.

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[4] Van Allen, C., and Jung, T., *J. Thoracic Surg.*, 1931, **1**, 3.

6512

Serological Evidence on the European Type of Typhus Fever in China.

CHAO-JEN WU. (Introduced by F. R. Dieuaide.)

From the Department of Medicine, Peiping Union Medical College.

Fletcher and Lesslar[1] described what they called tropical typhus from the Federated Malay States. By using several strains of *B. proteus* X for the Weil-Felix test they showed that, though clinically the same, urban cases gave a positive agglutination with the X_{19} (Warsaw) strain while those from rural districts gave positive tests only with the Kingsbury strain. Spencer and Maxcy[2] using the variants of X_{19}, X_2, and X_K (Kingsbury) found that the sera of patients with endemic typhus invariably agglutinated to a high titre the X_{19} strain, but possessed no appreciable group agglutinins for other strains. With Rocky Mountain spotted fever, however, X_{19} organisms were agglutinated significantly only in a minority of cases and to a lower titre than in endemic typhus; moreover, agglutinins for X_2 and X_K were also found in a considerable proportion of these cases. Felix and Rhodes[3] have recently studied more extensively the sera of patients with typhus and typhus-like diseases from various parts of the world and confirmed the above findings. There seems no doubt that some endemic diseases clinically simulating typhus give positive Weil-Felix reactions with the X_K strain to high or low titres, but not with the usual X_{19} strain, while still others give negative tests with any known strain. Felix and Rhodes propose to consider the various forms of typhus and typhus-like diseases as serological types of typhus. According to them the typhus nature of those diseases which give negative Weil-Felix reactions

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[1] Fletcher, W., and Lesslar, J. E., *Bull. Inst. Med. Res.*, Kuala Lumpur, F. M. S., 1925, 2; 1926, 1.

[2] Spencer, R. R., and Maxcy, K. F., *Pub. Health Rep.*, *U. S. A.*, 1930, **45**, 440.

[3] Felix, A., and Rhodes, M., *J. Hyg.*, 1931, **31**, 225.

TABLE I.
Agglutination of various *B. proteus* X strains by sera of typhus fever patients.

B. proteus strain	OX_{19}	HX_{19}	OX_2	HX_2	OX_K	HX_K
Group A.	800	200	0	0	0	0
Cases from	800	400	0	0	0	0
scattered parts	3200+	800	0	0	0	0
of the city (Peiping)	3200+	800	0	0	0	0
	400	200	0	0	0	0
	800	100	0	0	0	0
	400	100	0	0	0	0
	3200+	800	0	0	0	0
	400	0	0	0	0	0
	3200+	1600	0	0	0	0
	1600	400	0	0	0	0
	3200+	1600	0	0	0	0
	1600	400	0	0	0	0
	3200+	3200	0	0	0	0
	3200	400	0	0	0	0
	200	0	0	0	0	0
	400	0	0	0	0	0
	200	0	0	0	0	0
	800	100	100	0	0	0
	3200+	800	0	0	0	0
	200	200	0	0	0	0
	3200+	3200+	0	0	0	0
	3200+	3200+	0	0	0	0
Group B.	1600	800	100	0	0	0
Cases from an	1600	800	0	0	0	0
orphan home	3200+	800	0	0	0	0
	800	200	200	200	0	0
	800	400	200	200	0	0
	3200+	800	0	0	0	0
	1600	400	0	0	0	0
	1600	200	0	0	0	0
	1600	800	0	0	0	0
	1600	400	200	0	0	0
	200	100	0	0	0	0
Group C.	1600	800	0	0	0	0
Cases from a	1600	800	200	100	0	0
factory for	1600	800	0	0	0	0
poor children	3200+	1600	100	0	0	0
	800	200	0	0	0	0
	1600	400	100	0	0	0
	3200+	800	200	100	0	0
	1600	1600	0	0	0	0
	800	800	0	0	0	0
	3200+	3200+	0	0	0	0
	200	200	0	0	0	0
	800	400	0	0	0	0

but otherwise are typhus-like may later be proved when corresponding types or variants of *B. proteus* X are isolated.

Typhus fever as it occurs in Peiping is clinically of European epidemic type.[4] To determine whether it shows other agglutinins than those for the usual X_{19} and to detect the possible occurrence of hitherto undiagnosed typhus-like diseases which might co-exist in this city, the Weil-Felix reaction, using the "O" and "H" variants of X_{19}, X_2, and X_K,* was done in 46 cases of typhus fever and 16 cases of non-typhus fevers seen during the same season. All these cases were treated in the general medical wards of the Peiping Union Medical College Hospital from November, 1931, to May, 1932. The ordinary macroscopic agglutination technic with formalinized antigen suspensions was used. The tubes were immersed in a water bath at 37° C. for 2 hours and read after standing overnight in the ice-box. The lowest final dilution of the serum employed was 1:100 and the highest 1:3200. In many cases more than one test was done at different stages of the disease. The titre represented in Table I is the highest obtained for that particular case.

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[4] Cheer, S. N., and Dieuaide, F. R., *Nat. Med. J. China* (Chinese text), 1929, **15**, 355.

For the sake of clarity the results from the typhus fever cases are divided into 3 groups (Table I). All these cases invariably gave positive agglutination with the X_{19} strain, showing a higher titre for "O" than "H" antigen. Furthermore a few patients from an orphan home and a factory for poor children also showed group agglutinins for X_2 variants to a low titre. In no instance were we able to demonstrate agglutinins for X_K variants. This is in complete accordance with what Felix found in European typhus. Among the 16 cases of non-typhus fevers 3 agglutinated OX_{19} to the titre of 1:100 and the rest showed absolutely no agglutinins for any of the variants used.

In conclusion, it can be stated that typhus fever as it occurs in this part of China is of European or epidemic form serologically as well as clinically. No other typhus-like diseases have been encountered.

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* We are indebted to Dr. A. Felix of Lister Institute, London, who kindly supplied us all the strains used in this work.

6513

The Relative Proportion of Different Types of Cells in Blood and Lymphoid Organs of Normal Rats.

C. H. HU AND K. Y. CH'IN. (Introduced by S. H. Liu.)

From the Department of Pathology, Peiping Union Medical College.

Lymphoid cells, especially the small ones, fixed and stained by the ordinary methods, do not show sufficient morphological differences to be classified according to their relative maturity as is possible with the granular leucocytes. Difference of opinion thus exists over the developmental potency of the small blood lymphoid cells, and the exact stage of development of the small tissue lymphoid cells is not definitely determined. No further progress in our knowledge of these cells is likely to be made by the old methods of fixation and staining for study.

Stained with neutral red and janus green supravitally, however, the lymphoid cells present some very important morphological features which may be used as a basis for their finer differentiation. These features are: (1) the degree of opacity of the cytoplasm, (2) the number and size of mitochondria, and (3) the number of neutral red bodies.

We have been able to divide the lymphoid cells, irrespective of their sizes, into 3 types, namely: lymphoblasts, intermediate forms, and lymphocytes. The lymphoblasts have opaque cytoplasm, abundant, coarse mitochondria but no, or very few, neutral red bodies. The lymphocytes have clear cytoplasm, fine, scanty mitochondria, but numerous neutral red bodies. The intermediate forms, as their name implies, are morphologically intermediate between the lymphoblasts and lymphocytes, and are therefore presumably older than the lymphoblasts but younger than the lymphocytes.

This classification is used in the present supravital study of the lymphoid cells of the blood, spleen and lymph nodes of a large number of normal rats. The relative proportion of cells is fairly constant in all the animals studied. In Tables I and II the average values of the counts on the blood of 20, and those on the lymphoid organs of 7 animals are presented.

It is seen that both the blood and the lymphoid organs of normal rats contain lymphoblasts of all sizes. The peripheral blood, however, contains a higher percentage of mature lymphocytes, while in the lymphoid organs the intermediate forms predominate.

The presence in the rat blood of the lymphoid cells of all ages

TABLE I.

Average percentage values of the differential counts on the lymph nodes and spleen of 7 normal rats. 500 cells counted in each preparation.

Type of Cell		Organ	
	Inguinal lymph node	Mesenteric lymph node	Spleen
Small lymphoblasts	0.6	1.3	2.0
Small intermediate forms	75.7	64.7	60.0
Small lymphocytes	0.9	1.9	1.9
Small degenerated cells	21.2	29.3	28.9
Medium-sized lymphoblasts	0.3	0.4	1.3
Medium-sized intermediate forms, lymphocytes and degenerated cells*	0.3	1.0	2.7
Large lymphoblasts	0.2	0.4	0.8
Large intermediate forms, lymphocytes and degenerated cells*	0.2	0.3	0.9
Plasma cells	0	0.4	0.3
Monocytes	+	0	0
Clasmatocytes	0.1	0.1	0.6
Neutrophilic leucocytes	0.1	0.1	0.4
Eosinophilic leucocytes	+	+	0.3

+ = present, but less than 0.1%.

* Owing to their scarcity these cells are placed in one group under their respective sizes.

TABLE II.

Average percentage of 6 differential counts of the white blood cells of 20 normal white rats.

Small lymphoblasts	0.85	Degenerated cells, all sizes	0.15
Small intermediate forms	34.88	Monocytes	2.33
Small lymphocytes	23.11	Plasma cells	0.02
Medium-sized lymphoblasts	0.90	Clasmatocytes	0.02
Medium-sized intermediate forms	2.34	Myelocytes	0.07
Medium-sized lymphocytes	1.90	Poly. neutrophiles	26.58
Large lymphoblasts	1.33	Poly. eosinophiles	1.70
Large intermediate forms	2.65		
Large lymphocytes	1.17		

has led one of us (C. H. H.) to examine the blood of a few normal rabbits and normal human beings. It was found that a similar classification of their lymphoid cells is also possible and that lymphoblasts of all sizes are likewise present. The finding of both lymphoblasts and lymphocytes in the blood probably explains the controversy regarding the developmental potency of the small blood lymphoid cells. It seems quite possible that the presence of the lymphoblasts has led some to the conclusion that all "lymphocytes" are primitive cells and that of the mature lymphocytes has led others to the opposite conclusion that all "lymphocytes" are end cells.

6514

Development of Lymphocytes and Plasma Cells in Rats Experimentally Infected with Trypanosomiasis.

C. H. HU and K. Y. CH'IN. (Introduced by S. H. Liu.)

From the Department of Pathology, Peiping Union Medical College.

In a previous histological study of the spleen and the mesenteric and mediastinal lymph nodes of white rats experimentally infected with *Trypanosoma brucei*[1] (intraperitoneal injection of about 10,000 parasites), it was found that there was a marked increase of large and medium-sized cells with big irregular nuclear chromatin masses and abundant basophilic cytoplasm. These cells, owing to the large numbers of mitotic figures they contained, were considered as lymphoblasts.

In the sections of these organs of the infected animals killed at different stages of infection the histological pictures were found to vary according to the duration of the infection. There was no change at the end of first day of infection; at the end of second day many large lymphoblasts appeared, apparently as a result of hypertrophy of some of the small lymphoid cells. There were at this stage many transitional cells between the lymphoblasts and the small lymphoid cells. During the next 2 or 3 days, there was a progressive increase in number of both the large and the medium-sized lymphoblasts, while the number of the small lymphoid cells was on the decrease. Mitotic figures among the lymphoblasts became very numerous. The plasma cells and the transitional cells between the lymphoblasts and plasma cells also began to appear in the lymph follicles in large numbers. During the entire course of infection the plasma cells normally present in the medulla of the mesenteric and other lymph nodes did not show any morphological change, even though the cortex of the same nodes presented a picture of marked lymphoblastic hyperplasia.

To gain a better understanding of the findings in the histological study and to explain why only a certain number of the small lymphoid cells in the lymphoid organs can form large lymphoblasts by hypertrophy while others remain unchanged, supravital studies were made on the lymphoid organs and blood of a large number of normal and infected animals.

The results indicate (1) that the small lymphoid cells which can hypertrophy are really the small lymphoblasts, and those which cannot hypertrophy are the intermediate forms and the small lymphocytes, (2) that in the lymphoid organs of the infected animals there is an increase of lymphoblasts having the supravital staining characteristics mentioned in the previous communication,[2] (3) that mitotic figures are present only in the large and medium-sized cells having the characteristics of lymphoblasts, (4) that plasma cells which are greatly increased in the latter part of infection are derived from lymphoblasts, and (5) that in the blood of the animals at certain stages of infection there was also an increase of the lymphoblasts and occasional presence of plasma cells.

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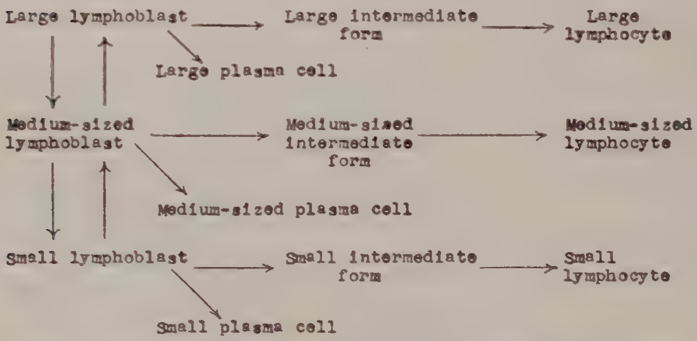
[1] Hu, C. H., "The Lymphatic Reaction in Experimental Trypanosomiasis." *The Nat. Med. J. of China*, 1931, **17**, 435.

From the results thus far obtained, one may represent the developmental relationship among the different types of lymphoid cells and plasma cells by the following schema:

SCHEMA



Arrows horizontal = Maturation
" oblique = Transformation
Arrows downward = Cell division
" upward = Hypertrophy

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[2] Hu, C. H., and Ch'in, K. Y., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 433.

6515

Oxygen Physically Dissolved in Blood Cell Suspensions and Hemoglobin Solution.

EN FU YANG. (Introduced by A. Carruthers.)

From the Department of Biochemistry, Peiping Union Medical College.

In Bohr's method[1, 2] for calculating the oxygen physically dissolved in blood or hemoglobin solution the assumption is made that the solubility of the gas in these fluids is as it is in water. In actual practice nitrogen is determined in blood or hemoglobin solution and from this and the solubility of oxygen in water the amount of oxygen physically dissolved in blood or hemoglobin solution is calculated. Direct determination of the oxygen in suspensions of cells or solutions of hemoglobin which have previously been treated with sodium nitrite, after which treatment it is supposed[3] hemoglobin no longer combines with oxygen, shows that the oxygen is greater than is accounted for in Bohr's method of calculation. Assuming the same physical properties for hemoglobin after treatment with nitrite it would appear that a correction should be made in the usual determinations of oxygen chemically combined with hemoglobin.

Treatment of cells or hemoglobin. (1) *Cells:* A mixture of 2 volumes of 0.9% sodium chloride solution and 8 volumes of 1.05% sodium nitrite was added to 10 volumes of fresh sheep's blood cells. The mixture was allowed to stand in the cold for half an hour and centrifuged. The cells were washed 3 times with cold van Dyke-Hastings solution[4] containing 0.60 gm. sodium cyanide per litre. (2) *Hemoglobin:* Cells prepared as above were electrodialyzed and the concentrated hemoglobin solution treated with alumina cream to remove the stroma. Solutions containing 20% hemoglobin could be obtained.

To show that oxidation in the cell preparation was complete, (1) nitrite treated cells were suspended in an equal volume of normal saline mixture saturated with air and 5 cc. were introduced into the van Slyke gas chamber. Five cc. of air saturated ferricyanide solution[5] were added and the oxygen determined. The oxygen from this determination was the same as the oxygen found in a sample

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[1] Bohr, C., *Compt. rend. Acad.*, 1897, **124**, 414.

[2] Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, **49**, 1.

[3] Haldane, J., Makgill, R. A., and Mavrogordato, *J. Physiol.*, 1897, **21**, 160.

[4] Miller, C. P., Jr., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 748.

[5] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

of cells mixed with ferricyanide outside of the chamber. (2) Samples of cells were treated with equal volumes of isotonic solutions of various nitrite concentrations and allowed to stand at room temperature for half an hour. When the nitrite concentration was higher than 0.5% the oxygen content showed no change.

Equilibration and analysis: Samples of cells or hemoglobin were equilibrated with various tensions of oxygen at 38°C. in a pair of double tonometers, the gas constantly released to atmospheric pressure and after the pressure became constant equilibration was continued for half an hour. Gas and fluid were separated in the usual manner. Oxygen in the fluid was determined with van Slyke's manometric apparatus,[5] 5 cc. samples being used for each determination, and oxygen in the gas phase was determined with Haldane's gas apparatus.[6] The total solid in the cell or hemoglobin preparations was determined by drying 5 cc. samples over night at 110°C.

Results. The relationship between concentration of cells or

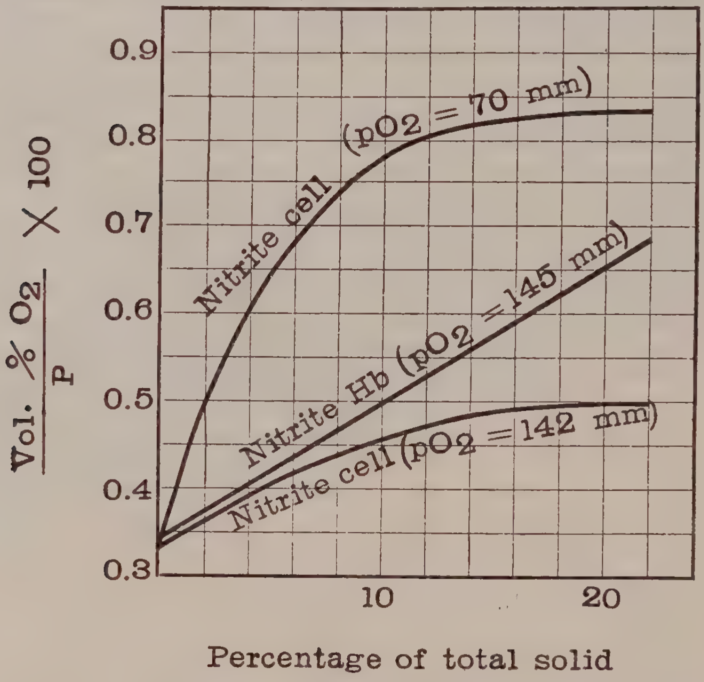

Fig. 1

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[6] Henderson, Y., *J. Biol. Chem.*, 1918, **33**, 21.

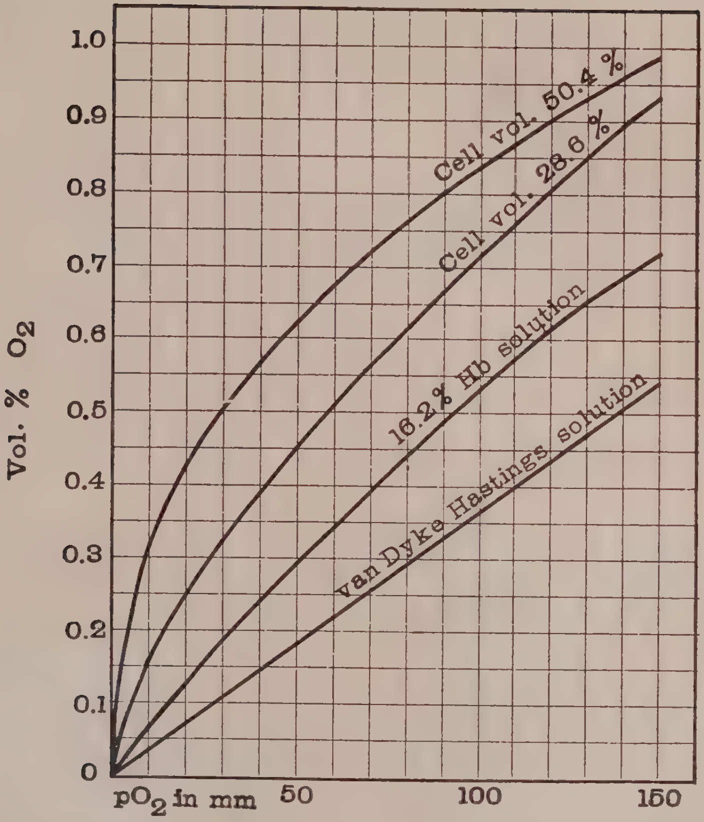


Fig. 2

hemoglobin and oxygen is shown in Fig. 1. For nitrite cells there is an increase in oxygen content up to 50% of cells by volume (*i. e.,* total solid of 16%) but above this level there appears to be no further increase. With nitrite hemoglobin solutions there is an increase in oxygen proportional to the increase of solid matter up to 20%.

Effect of gas tension: Fig. 2 shows that when cells are suspended in an equal volume of van Dyke-Hastings solution and equilibrated with gas of various oxygen tensions the ratio of oxygen in the gas phase to that in the liquid phase decreases with increase in partial pressure of oxygen. Individual preparations of cells give detectable variations, but the shape of the curve is unchanged. With nitrite hemoglobin solution the change in ratio at varying oxygen tensions is slight.

Cleveland Section

Institute of Pathology, Western Reserve University,
December 9, 1932.

6516

Excretion of Bismuth in Series of Clinical Bismuth Treatments.

TORALD SOLLMANN, H. N. COLE AND KATHARINE I. HENDERSON.
From Western Reserve University School of Medicine.

The excretion was studied quantitatively, usually on 3 patients for each compound, with 3 methods of administration: *Single*—one injection; *weekly*—one injection per week for 3 weeks; *multiple*—several (generally 3) injections per week for 3 weeks. In each series, the study was continued through an after-period of 2 weeks, a few patients were studied longer.

Compounds studied and bismuth dosage: (A) Water-soluble in water: Sodium bismuth citrate and sodium bismuth tartrate, 32 mg.; water-soluble in ethylene glycol: Sodium bismuth citrate, 32 mg. (B) Water-soluble suspended in oil (Sodium potassium bismuth tartrate), 32, 48, and 64 mg. (C) Water-insoluble suspended in oil (Bismuth subsalicylate), 75 mg. (D) Oil solutions: Quiniobine (Quinine bismuth iodide dissolved in olive oil with lecithin, 60 mg.; Bismo-cymol (Bismuth camphocarboxylate), 100 mg.; Biliposol (Bismuth ethylmethylnonoate), 80 mg. *All doses refer to the bismuth content.*

General Conclusions: 1. The *resemblances* between these bismuth compounds appear more impressive than the differences; the salicylate stands apart, with a much slower but more persistent absorption and excretion. 2. The *excretion and absorption velocity* is in the order of: Watery solutions (A) > oil solutions (D) > tartrate oil (B) > salicylate oil (C). 3. The *peak excretion and the total (cumulative) excretion* (within 3 weeks) run fairly parallel in the general order:

Water-soluble (A) multiple \ oil solutions (D) \ tartrate oil (B) \
Oil-sol. (B) ” / weekly / weekly /
oil solutions (D) \ water-soluble (A) \ tartrate oil (B) \
single / weekly / single /
water-soluble (A) \ salicylate oil (C)
single / single

4. The *percentage excretion* is nearly uniform for practically all, 15 to 25% within 3 weeks; it is much lower for salicylate-oil—2.9% (but here the excretion is very persistent, and longer after-period would give relatively higher percentage). The percentage excretion is materially above the average with single oil-solutions (D)—39 and 45%.

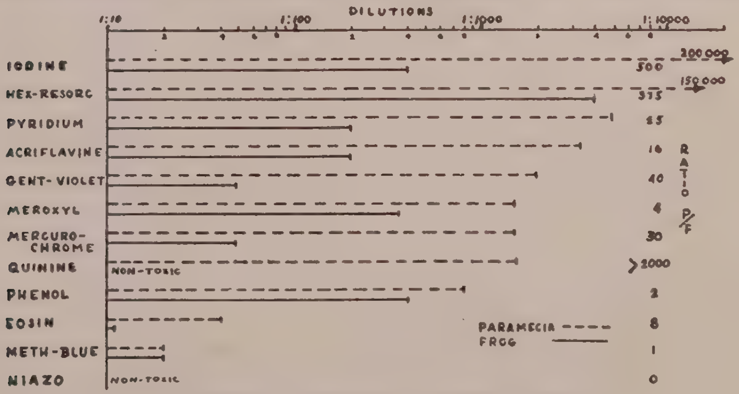
5. The *fecal excretion*, so far as studied, seems to be about a tenth of the urinary excretion and fairly parallel to it.

6517

Cell Injury by Antiseptics.

VICTOR A. BADERTSCHER. (Introduced by Torald Sollmann.)
From Western Reserve University School of Medicine.

Microscopic observation of the duration of the ciliary motion of the pharyngeal epithelium was utilized as an indicator of progressive injury to mucous membranes. The ciliary motion of infusorial cells (paramecia) was studied for comparison. The chart shows the median "effective dilutions"; namely, for the frog, the concentration required to stop within 20 to 40 minutes the ciliary motion



DILUTIONS OF ANTISEPTICS EFFECTIVELY STOPPING CILIARY MOTION IN FROG TISSUE AND PARAMECIA
FIG. 1.

in small squares of mucosa clipped from the pharynx; for the paramecia cultures, the arrest period was chosen as 4 to 5 minutes. The scale of dilutions on the chart is logarithmic. The preparations are seen to be rather markedly toxic. The numbers to the right of the chart represent the ratio of effective paramecia dilutions to effective frog dilutions and are somewhat of an index of selectiveness of the antiseptics to the 2 preparations. Methylene blue was about equally toxic to both. With all the other antiseptics, the paramecia were more susceptible than the pharyngeal epithelium; the ratio being from 2 to 8 for phenol, meroxyl, and eosin; from 16 to 40 for acriflavine, pyridium, mercurochrome and gentian violet; 375 to 500 for hexylresorcinol and iodine; and infinite for quinine.

Illinois Section.

University of Chicago, December 16, 1932.

6518

Utilization of Certain Sugars and Their Derivatives by Bacteria.

STEWART A. KOSER AND FELIX SAUNDERS.

From the Departments of Hygiene and Bacteriology and of Physiological Chemistry, University of Chicago.

In the experiments reported here we have studied the utilization by bacteria of a number of derivatives of the commoner sugars. The sugars used comprised several methyl glycosides, an amino sugar, a sugar acid, a sulphur-containing sugar, and 2 heptoses. The availability of these unusual sugars was compared with that of the common sugars from which they were derived.

All sugars were sterilized by filtration and added to suitable culture media to give a concentration of 0.5 to 1.0%. Each lot of medium containing a test sugar was then inoculated with about 25 representative species of the commoner bacteria. Fermentation of the sugar was determined by the usual methods of detecting acid and gas production.

It was found that when the hydrogen of the hydroxyl group attached to the number one carbon of the sugar molecule was replaced by a methyl group, the resultant modified sugar was distinctly more resistant to bacterial attack. Thus, of many bacteria which fermented glucose, only a few were able to make use of alpha methyl glucoside. Those able to ferment the methyl glucoside included: *Proteus, Bact. friedländeri, Bact. aerogenes,* pneumococci, several hemolytic streptococci, and a yeast.

Similar comparative tests with mannose and alpha methyl mannoside showed that the latter was not fermented by any of the organisms capable of using mannose. Two methyl pentoses, beta-methyl-l-arabinoside and beta-methyl-d-xyloside, were not used by those organisms capable of fermenting l-arabinose and d-xylose.

Two sugars containing 7 carbon atoms, alpha glucoheptose and alpha glucoheptulose, were not fermented by any of the organisms. Glucosamine was used by most of the cultures which fermented glucose, with the exception of *Proteus* and 2 yeasts which gave negative results. Gluconic acid was also fermented by most of the glucose-splitting types, with the exception of the streptococci, pneumococci and the yeasts. Glucose ethyl mercaptal, a sulphur containing sugar, gave entirely negative results.

6519

A Microcrystallographic Study on Phosphates and its Practical Application.

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In precipitating phosphate solutions of a concentration of 1/20 to 1/200 of their molecular weight in a solution of the specific gravity of the urine, by using an ammonium magnesium sulphate reagent (Dowd's reagent), we find 8 different forms of crystals, changing gradually at certain amounts of the phosphorus. The form of these crystals can be used for an estimation of the amount of phosphorus in the urine.

Three cc. of the urine and 0.6 cc. of the reagent (10% solutions of magnesium sulphate, ammonium chloride, ammonium hydroxide, 20 cc. of each) are mixed and the crystals, which are formed after at least 10 minutes, are examined with the 4 mm. objective. The predominant crystal indicates the amount of phosphorus. By titrating dilutions of the urine in a 2.5% solution of sodium chloride and precipitating 3 cc. of the dilutions with 0.6 cc. of the reagent the amount of phosphorus in the urine can be calculated from the last dilution, in which the crystal type of the undiluted urine appears. (Crystal type I corresponds to 103 mg. of P in 100 cc. of urine, type II, 93 mg., and so on; type VIII indicates that there are less than 10 mg. of P in 100 cc.) The specific gravity of the urine or the 24 hour specimen may be used for the calculation of the total output of phosphorus during one day. The presence of considerable amounts of urea or of sugar change the form of the crystals in a typical way.

6520

A Comparative Study of the Proteins of Citrus Seeds.

A. A. BASS AND FELIX SAUNDERS. (Introduced by F. C. Koch.)

From the Department of Physiological Chemistry, University of Chicago.

The seeds of citrus plants are particularly adapted to comparative studies of the proteins of different plant species. They can be obtained relatively easily and yield a crystalline globulin which has been given the name "pomelin". To isolate the protein the seeds are ground to a fine meal and the oil, which constitutes about 35% of the total weight, is removed with benzene. The defatted meal is covered with 10 times its weight of a salt solution which has been heated to 55°. (The concentration of the salt solution may vary from normal to saturation.) The salt solution is recovered by filtration or centrifuging and saturated with ammonium sulfate. The precipitated protein is collected by filtration and redissolved in a minimum of water. If the solution is not clear any sediment is removed in the centrifuge. The solution is dialyzed in viscose bags for 48 hours against cold (6-10°) running distilled water which causes the globulin to separate in a crystalline form. No preservative is used. The protein is collected and washed with several changes of alcohol starting with 30% and running up to absolute. After a final washing with absolute ether and air-drying, the protein is ready for analysis.

TABLE I.

Nitrogen Distribution of Protein (Pomelin) from Various Citrus Seeds.

	Orange	Lemon	Grapefruit
Total N	16.29	16.44	16.21
Amino N	72.59	77.08	78.71
Amide N + Humin N	14.95	15.58	15.76
Di-amino N	12.14	12.48	13.20
Mono-amino N	52.28	52.31	51.84
Non-amino N	——	6.04	5.54
Total S—	0.40	0.62	0.50

The results of the analysis for various nitrogen fractions are shown in Table I. The analytical figures show that the pomelin from grapefruit, lemon and orange is the same in each case. The analysis of pomelin from tangerine seeds also agrees with these figures although there was not enough material available for complete analysis. The crystal form as far as can be seen under the microscope is the same in each case. The authors conclude that the seeds from the citrus plants: grapefruit, lemon, and orange, and probably tangerine, all yield the same crystalline globulin, pomelin.

6521

Influence of Age on Amount of Normal Agglutinins in the Blood of Cattle.

EDWIN O. JORDAN.

From the Department of Hygiene and Bacteriology, University of Chicago.

The currently accepted explanation of the frequent presence in normal human adults, as contrasted with young children, of antibodies such as diphtheria antitoxin and the substance that neutralizes the virus of poliomyelitis, is that the antibodies have been specifically generated by an unnoticed or a subclinical infection. The occurrence of normal bacterial agglutinins in the blood of various adult domestic animals, as demonstrated by Burgi, Gibson and others, has suggested some observations upon the influence of age on the presence and abundance of these antibodies. These agglutinins are common in the blood of cattle, they are specific at least to the extent of being removed by absorption with specific bacterial cells and they comprise both the H and O antigenic types.

The standard macroscopic agglutination technique was used. The serum was freshly obtained from cattle brought to the Chicago stockyards, and was from 3 groups: (a) calves (under 1 year); (b) heifers (approximately 1-2 years); (c) "cows and steers". The table shows, as observed by others, that there is considerable variation in the amounts of agglutinins for different bacterial strains, and without exception, agglutination is greater, often in a very striking degree, in the serum of the older animals than in that of the calves. The animals classed as heifers appear to have about the same amount of bacterial agglutinins in their blood as the older animals, but the titers average somewhat lower. There also appears to be a sex difference, for simplification's sake not indicated in the table. The serum of females contains on the average a distinctly greater amount of agglutinins than that of males.

Some observers have used strains of the Salmonella group to test for the presence of normal agglutinins.[1] Since several members of this group, notably *S. aertrycke* and *S. enteritidis* are natural infecting agents in cattle and since overlapping of *salmonella antigens* is commonly observed, the results obtained by the use of these strains are open to more than one interpretation. On the other hand, the agglutination reactions obtained with cholera vibrios

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[1] Lovell, R., *J. Comp. Path. and Ther.*, 1932, **45**, 27.

TABLE I.
Agglutination by Normal Bovine Sera.

Species	No. sera tested		Not agglutinating at 1:40	Maximum Titer			
				1:40	1:100	1:200	1:400 or higher
B. coli (human feces)	Calves	10	10	0	0	0	0
	Heifers	23	21	2	0	0	0
	Cows and steers	24	17	4	3	0	0
*B. aerogenes** (isolated from soil apparently non-contaminated)	Calves	41	40	1	0	0	0
	Heifers	23	0	5	13	5	0
	Cows and steers	57	0	10	30	17	0
B. dysenteriae Flexner	Calves	41	40	1	0	0	0
	Heifers	23	0	5	7	10	1
	Cows and steers	52	0	2	28	19	3
B. dysenteriae Shiga	Calves	20	20	0	0	0	0
	Heifers	23	13	9	1	0	0
	Cows and steers	21	9	12	0	0	0
B. dysenteriae Sonne	Calves	41	39	1	1	0	0
	Heifers	20	0	0	2	12	6
	Cows and steers	27	0	1	7	15	4
B. pyocyaneus	Calves	41	41	0	0	0	0
	Heifers	23	1	5	12	5	0
	Cows and steers	57	0	12	25	18	2
Vibrio cholera A	Calves	61	60	1	0	0	0
	Heifers	23	4	19	0	0	0
	Cows and steers	56	15	32	9	0	0
Vibrio cholera B	Calves	10	9	1	0	0	0
	Cows and steers	9	0	4	4	1	0
Vibrio metchnikovi	Calves	10	8	2	0	0	0
	Heifers	23	4	19	0	0	0
	Cows and steers	24	1	16	7	0	0

* Another strain of *Bact. aerogenes* isolated from human feces did not agglutinate at 1:40 with the serum of calves (41 specimens), heifers (20), or adult bovines (53).

and perhaps those with certain dysentery bacilli strains, as shown in the table, are not so readily to be referred to previous specific infection with these bacteria. It is evident that the older bovines contain in their serum specific bacterial agglutinins which are absent or very scanty in the young and which are not likely to have been generated by infection with the specific homologous bacteria.

6522

Nature of the Substance Causing Staphylococcus Food Poisoning.

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From the Department of Hygiene and Bacteriology, University of Chicago.

The difficulties experienced in studying staphylococcus food poisoning when the only available subject for experimentation was the human volunteer have been in part overcome by the observation that monkeys can be fed staphylococcus filtrates in such a way as to insure a certain proportion of definite and characteristic gastro-intestinal reactions.[1] Utilizing monkey feeding as a crude method of determining the presence or absence of the significant principle, some data bearing on the nature of this principle have been obtained. In all the tests here reported only animals showing actual vomiting have been set down as yielding a positive reaction; other symptoms such as pallor, diarrhea, and evident distress have been noted but not recorded as indicating an unmistakable positive result. Not all monkeys react to the active principle inducing vomiting and consequently, before using an animal for experiment we have fed it with a filtrate of known potency. Negative results were controlled by subsequent feeding with potent filtrate. The possible development of tolerance has also been controlled.

The following results have been obtained: 1. The active principle will not distill. 2. It is not readily dialyzable. 3. It is markedly unstable to N/100 NaOH. 4. It is unstable to heat in N/100 HCl solution. 5. It is not identical with the hemolytic substance present in many staphylococcus filtrates nor does it produce a skin reaction. 6. It is completely removed from acid aqueous solution by extraction with ethyl ether or chloroform as judged by our method of assay. 7. It may be extracted from alkaline solution with ethyl ether or chloroform but the deleterious effect of alkali tends to mask such removal.

Feeding with a neutral saline or aqueous solution of the ether or chloroform extracts from which all traces of the solvent had been removed by warming *in vacuo* caused typical vomiting in monkeys. The extracted residues caused no symptoms. Monkeys injected intravenously with 5 cc. of saline solution of acid ether extract of potent filtrate vomited and manifested the usual signs of acute distress of the gastro-intestinal tract but showed no other symptoms.

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[1] Jordan, Edwin O., and McBroom, Josephine, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 161; Dack, G. M., and Woolpert, O. C., *J. Inf. Dis.*, 1933

The same solution given intravenously to guinea pigs, rabbits, cats, and dogs produced no ill effects whatever.

6523

Neutralization of Poliomyelitis Virus by the Serum of Native Chinese of Peiping.*

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From the Department of Hygiene and Bacteriology, University of Chicago.

The studies of Frost,[1] Aycock,[2] and others on the epidemiology of acute anterior poliomyelitis have led to the opinion that the virus is more widely distributed than the incidence of clinical cases indicates. Aycock and Kramer[3] found that 86% of serums from 21 normal adult persons of Atlanta, Georgia, possessed virucidal properties; these results suggest that immunity as measured by the neutralization test is extensively present in the warm southern latitudes, as other investigations have shown in the cooler northern latitudes. Recent investigations into the incidence of neutralizing serums in tropical and subtemperate regions where clinical poliomyelitis is uncommon have extended the findings of Aycock and Kramer. Hudson and Litterer[4] observed that 84% of the serums of 25 normal adults of Nashville, Tennessee, were capable of neutralizing the virus; Soule and McKinley[5] found that the serums of 8 adult Porto Ricans without history of attack or exposure to the disease were virucidal in every instance; and we[6] recently reported that 18 out of 19 serums from normal Liberian negroes were able to inactivate the virus.

To obtain data on the extent of immunity in cooler climates where the occurrence of frank cases of the disease is seldom re-

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* This work was supported in large part by grants from the Milbank International Fund for the study of Infantile Paralysis.

[1] Frost, W. H., *Hyg. Lab. Bull.*, No. 90, 1913, Washington, D. C.

[2] Aycock, W. L., *J. Prev. Med.*, 1929, **3**, 245.

[3] Aycock, W. L., and Kramer, S. D., *J. Prev. Med.*, 1930, **4**, 201.

[4] Hudson, N. P., and Litterer, W., unpublished experiments.

[5] Soule, M. H., and McKinley, E. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 168.

[6] Hudson, N. P., and Lennette, E. H., paper read before the Epidemiological Section of the American Public Health Association, Washington, D. C., October 24, 1932. To be published.

ported, we turned to the examination of the serum of native Chinese. Zia[7] writes that "among 25,000 admissions to the Peiping Union Medical College Hospital, there has been only one diagnosis of this disease," but further reports that he recently observed several cases in the acute stage. He adds that, while it is unlikely that epidemics of poliomyelitis in China have been overlooked, it does seem probable that the apparent infrequency of sporadic cases may rest on failure to recognize the manifestations of the disease.

Serum specimens for the tests were furnished us through the courtesy of Dr. W. A. Sawyer of the International Health Division of the Rockefeller Foundation. Twelve similar specimens were examined by Jungeblut,[8] who informs us that 11 neutralized the poliomyelitis virus.

The technic employed was similar to the standard procedures in use and previously described.[9] Temperatures and observations on the test monkeys were recorded daily for 6 weeks after inoculation. Ten undiluted specimens were examined for their ability to neutralize the virus. The results with 2 are indeterminate, since the test animals succumbed to infections other than poliomyelitis 17 and 22 days after injection. The 8 monkeys, inoculated with the remaining samples mixed with virus, survived without fever or other symptoms. The virus control monkey died of the experimental disease 10 days after injection; the convalescent-serum control survived without exhibiting any symptoms of infection. Histological examination of the spinal cord of the virus control monkey revealed lesions typical for experimental poliomyelitis; this evidence was lacking in the central nervous system of the animals which died of intercurrent infections.

The donors—9 males and 1 female—were native Chinese who had resided in Peiping for from 3 to 30 years, and presumably had never been outside China. The age distribution was: one each of 26, 32, 35, 36, and 37 years, three 40 years, and one each of 43 and 46 years. These serums were among those reported by Hughes and Sawyer[10] as being devoid of any power to protect mice against infection with yellow fever virus. Since the presence of yellow fever in China has been definitely excluded, the failure of the serums in mouse-protection tests was what might be expected. On the other hand, although China is apparently free from poliomyelitis in epidemic form,[11] the high proportion of neutralizing serums occasions no surprise, inasmuch as previous contact with the specific virus cannot be ruled out. The few clinical cases of the disease reported are ample evidence that the virus is present in the population and consequently may serve to immunize it.

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[7] Zia, S. H., *Nat. Med. J. China*, 1930, **16**, 135.

[8] Jungeblut, C. W., personal communication. Preliminary report in "Poliomyelitis," Williams and Wilkins, Baltimore, 1932, 440.

[9] Hudson, N. P., and Lennette, E. H., *J. Prev. Med.*, 1932, **6**, 335.

[10] Hughes, T. P., and Sawyer, W. A., *J. Am. Med. Assn.*, 1932, **99**, 978.

6524

A Method for the Determination of Ethyl Alcohol.*

THEODORE E. FRIEDEMANN AND EARL B. RITCHIE.

From the Department of Medicine, University of Chicago.

Ethyl alcohol is either present as such or it may be formed under certain conditions by many plant and animal cells. It is the chief product of carbohydrate metabolism of non-pathogenic as well as pathogenic yeast-like organisms.[1] Smaller quantities have been found in bacterial and mold cultures. It is found also in blood (0.001 to 0.004%) and tissues (0.0007 to 0.0026%) of animals.[2]

The usual physical methods are not applicable to the small amounts found in biological materials, and for this reason chemical methods have been proposed and used with a fair degree of success. The methods of Nicloux[3] and Widmark[4] depend upon oxidation of the alcohol by $K_2Cr_2O_7$ in the presence of strong H_2SO_4. Varying degrees of oxidation are obtained, depending upon the condition.

In the method which we propose the oxidation is carried out in 2 steps by $KMnO_4$. It is first oxidized by hot alkaline permanganate (almost quantitatively) to oxalic acid. The latter is then completely oxidized on acidification with H_2SO_4. The low final acidity permits a more accurate iodometric determination of residual oxidizing agent.

The sample, previously deproteinized, is pipetted into a 300 cc.

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[11] League of Nations Monthly Epidemiological Reports, 1930, R. E. 135, 136.

* This study was aided by a grant from the Bartlett Memorial Fund of the University of Chicago.

[1] Friedemann, Theodore E., and Ritchie, Earl B., unpublished data.

[2] Gettler, A. O., Niederl, J. B., and Benedetti-Pichler, A. A., *J. Am. Chem. Soc.*, 1932, **54**, 1476.

[3] Nicloux, M., *Compt. rend. Soc. biol.*, 1931, **107**, 529.

[4] Widmark, E. M., *Biochem. Z.*, 1922, **131**, 473.

Kjeldahl flask. Talcum is added, and the volume is made up to about 150 cc. Eighty to 100 cc. are distilled into another 300 cc. Kjeldahl flask containing about 50 cc. of cold water. An apparatus similar to, but somewhat larger than that recently described by Bok,[5] is satisfactory. To remove acetone and aldehydes the distillate is treated with 10 cc. of a saturated $HgSO_4$ solution in N H_2SO_4 and 5 cc. of saturated NaOH. It is then slowly distilled until about 80 cc. have been collected. The volume is diluted to the 100 cc. mark and an aliquot is analyzed.

Fifty cc. of standard $KMnO_4$ (0.1 or 0.01 N) and approximately 10 cc. of 5N NaOH are placed into an Erlenmeyer flask. This is heated 10 minutes in the boiling water bath. The flask is momentarily removed and the solution containing alcohol is run in by pipette below the surface. Heating is continued for 30 minutes. The solution is cooled. It is acidified by 10 cc. of 10 N H_2SO_4 and allowed to stand a few minutes. An excess of KI is then added and the iodine is determined by standard thiosulfate. Blank determinations are run simultaneously. The difference between the blank and the unknown represents the volume of standard $KMnO_4$ used up by the oxidation. Within the following limits, and under the conditions described above, 1 mg. is oxidized by 2.38 cc. 0.1 N $KMnO_4$ (limits 0.4 to 8 mg.). 0.1 mg. is oxidized by 2.38 cc. 0.01 N $KMnO_4$ (limits 0.04 to 0.4 mg.).

The end points are very sharp. The maximum error is about ±3%.

6525

Rate of Ammonia Liberation in Tryptic and Peptic Digestion of Casein.

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The first properly controlled quantitative study of the rate of liberation of ammonia in tryptic digestion was publised by Hunter and Smith,[1] who discussed the early literature on this phase of the subject and that need not be repeated here. They found that in tryptic digestion ammonia was liberated very slowly and that amidolysis took place very much less rapidly than peptide hydrolysis.

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[5] Bok, J. C., *J. Biol. Chem.*, 1931, **93**, 645.

[1] Hunter, A., and Smith, R. Q., *J. Biol. Chem.*, 1924, **62**, 649.

In 10 days of digestion at the time when 65% of the total bound nitrogen was liberated, only about 25% of the total potential ammonia was liberated. The authors compared their results with those of Henrique and Gjoldback[2] and pointed out that whereas the trypsin used by the latter was much less active in peptide hydrolysis it was a good deal more potent in the liberation of amide groups. They concluded that amidolysis is probably due to a separate enzyme and that a pure trypsin might produce no ammonia from proteins.

Zunz,[3] in a quantitative study of the course of peptic digestion on casein and albumin, reported that a little more than one-third of the available ammonia in casein was liberated in 15 days. Henrique and Gjoldback[2] reported 7.2% in 30 days and 9% in 108 days No control of HCl + casein was shown. Effront[4] reported 5.0% in 15 hours and calls attention to the fact that 2-3 gm. HCl per liter eventually causes the formation of amide nitrogen. "The quantity of nitrogen thus formed is much smaller than is formed in the presence of pepsin hydrochloride, and very often this fact is not taken into account."

Having on hand highly active pepsin* and trypsin† preparations it was thought worth while to make a parallel study of the rate of liberation of ammonia by these enzymes.

Experiment I. An approximately 5% solution of Hammersten's casein was prepared by suspending 50 gm. in 500 cc. distilled water and adding N/10 NaOH or 1% Na_2CO_3 solution until completely dissolved. The pH of this solution was then adjusted to 8 and made up to volume. The solution was clear and it was not necessary to filter. The methods of sampling, concentration of enzyme, controls, preservatives, and incubation were those of Hunter and Smith.[1]‡

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[2] Henrique, V., and Gjoldback, J. K., *Z. Physiol. Chem.*, 1911, **75**, 363.

[3] Zunz, E., *Z. Physiol. Chem.*, 1898, **28**, 151.

[4] Effront, J., Biochemical Catalysts in Life and Industry, New York, John Wiley and Sons, 1917, 242.

* Pepsin prepared according to the method of Thomas L. Meekin (unpublished University of Chicago thesis, 1925) had an activity of 10X that of the Armour and Company U.S.P. pepsin 1-3000.

† Difco trypsin 1:110, commercial product, had the same activity as the purified product of Robert W. Bates (unpublished University of Chicago thesis, 1931). Activity comparison was made by the refractive index method and found to be 10X as active as the Parke, Davis and Company's pancreatin U.S.P.

‡ Conditions for aeration were established as follows: a peptone solution and an ammonium sulfate solution respectively were aerated for 30 minutes, one hour, and one and one-half hours. The results were identical and therefore the 30-minute aeration was used throughout this work.

TABLE I.

Cross results:	Cas. digest*		Cas. control % total N as		Net results % total N as		Author % total hydrolysis		Hunter and Smith	
	NH_2 D	NH_3 D	NH_2 C	NH_3 C	NH_2 D-C	NH_3 D-C	Peptide groups	Amide groups	Peptide groups	Amide groups
Orig. solution			4.17	0.075†						
After 1 hr.	33.16	0.32	4.25	0.075	28.99	0.245	42.53	2.41	44.8	2.6
” 24 hr	42.12	0.50	4.59	0.20	37.53	0.30	55.2	2.95	56.0	7.7
” 5 days	48.05	2.24	6.26	0.44	41.79	1.80	61.46	17.74	63.2	21.2
” 10 days	50.71	3.15	8.71	0.96	42.00	2.19	61.78	21.59	65.4	25.2
” 88 days	56.40	5.88	14.51	2.12	41.89	3.76	61.62	37.07	64.2	40.5
” 144 days		6.50		3.10		3.40		33.52		
Complete hydrolysis by acid			72.15	10.45	67.98	10.38				

* Enzyme control is not shown but correction was made.

† The figures are averages of triplicate determinations. In one case an additional 0.25 gm. of trypsin was added, but no effect was noted.

In comparing the net results obtained with those taken from Hunter and Smith, it is apparent that although the percentages are somewhat lower both in peptide and amide hydrolysis, yet in general the data are confirmatory. The trypsin preparation used had the same order of proteolytic and amidolytic activity as that used by Hunter and Smith. As pointed out by the former authors the amide hydrolysis falls far behind the peptide hydrolysis not only in absolute velocity but in the rate with which the hydrolytic equilibrium is approached. The proteolytic activity is greatest in the first hour and reaches an equilibrium in 5 days (61.46%, and in 10 days, 61.78%) amino nitrogen is liberated, after which time the increase may be accounted for by the spontaneous hydrolysis of the substrate in the control. On the other hand, the enzymatic production of ammonia continues slowly but constantly. The 88th day marks a gain of 52% over the 5th day. Thus on the 88th day, 37% (corrected) of the total potential amide nitrogen was liberated. An apparent equilibrium was attained after that. The spontaneous amidolysis of the substrate in the control on the 144th day was greater than in the digest. If calculated without correcting for the protein control of the total available ammonia nitrogen, 62.2%, (or two-thirds) is obtained and one-third is probably in a trypsin-stable complex reported by Luck.[5]

Experiment II. The rate of liberation of ammonia in tryptic and peptic digestion was followed simultaneously: the same concentrations of enzyme and substrate were used. No measures were taken to stop hydrolysis in the case of pepsin. Aliquot portions were taken, allowed to come to room temperature and then quantitative measurements were made for analysis.

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[5] Luck, J. M., *Biochem. J.*, 1924, **18**, 679.

A. 1. 5 gm. casein suspended in 250 cc. N/10 HCl + 0.125 gm. purified pepsin.§
   2. 5 gm. casein suspended in 250 cc. N/10 HCl + 0.0 gm. purified pepsin.
B. 1. 5 gm. casein dissolved in N/10 NaOH neutralized with HCl and adjusted to same pH as A.1 + 0.125 gm. purified pepsin.
   2. Same as B.1, but no pepsin.
C. 1. 5 gm. casein dissolved in 250 cc. 0.4% Na_2CO_3 + 0.125 gm. trypsin.[4]
   2. 5 gm. casein dissolved in 250 cc. 0.4% Na_2CO_3 + 0.0 gm. trypsin.

TABLE II.
% of total nitrogen as ammonia nitrogen.

Time of Digestion	A 1	A 2	A 3(1-2)	B 1	B 2	B 3(1-2)	C 1	C 2	C 3(1-2)
5 hr.	0.05	0.05	0	0.04	0.05	—0.01	0.10	0.05	0.05
24 hr.	0.50	0.50	0	0.719	0.719	0.0	0.47	0.05	0.42
7 days	3.36	0.983	2.37	4.31	1.95	2.36	2.43	0.62	1.71
16 days	5.32	3.10	2.22	6.60	3.96	2.64	2.69	0.89	1.80
25 days	6.16	4.29	1.87	7.25	5.50	1.75	4.14	1.70	2.44
52 days	7.71	6.21	1.50	8.12	7.19	0.93	5.03	2.28	2.75
83 days	8.28	7.34	.94	8.84	8.53	0.31	6.01	3.10	2.91
109 days	8.95	8.04	.91	9.45	6.63	0.82(?)	7.05	3.78	3.27
120 days	9.65	9.05	.60				7.10	3.88	3.22

The only difference in A and B was that the casein in B was first dissolved in NaOH and then neutralized and adjusted to the same pH as A. This treatment rendered the casein more flocculent and, as the results indicate, the liberation of ammonia is slightly more rapid both in the control and in the digest as compared with A.

Table II shows that although pepsin hastened the liberation of ammonia from casein, N/10 HCl at 37°C. will liberate practically the same amount if sufficient time is allowed. Thus in 120 days the difference between pepsin-HCl and HCl alone is 0.60%. The effect of pepsin begins to decrease after the 7th day. In the case of trypsin (column C) the course is quite different. Trypsin at pH 8 definitely liberated ammonia from casein (C-1) more rapidly than was liberated by pH 8 and no trypsin (C-2). An apparent equilibrium was reached in 109-120 days when no more ammonia was liberated in either flask and at this point the ammonia concentration in C-1 was almost twice that in C-2, thus showing definitely an enzymatic effect. The data with pepsin may be interpreted that pepsin had no amidolytic activity. The catalytic effect may be due to the proteolysis, *i. e.*, during peptic hydrolysis nitrogenous compounds were formed which gave off ammonia more easily under the conditions of the experiment or "internal anhydrides"[6] might have opened exposing amide groups which were hydrolyzed more easily by the HCl. In the control tube with no peptic hydrolysis the action of HCl took longer. Thus while the effect of pepsin might be explained on the basis of the physical consistency of the substrate and the proteolytic properties, these factors did not enter into the case of trypsin. As was pointed out in Experiment I, proteolysis ceased although amidolysis continued till equilibrium was established when about two-thirds of the amide groups were split off.

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§ Enzyme controls were run and corrections for ammonia in pepsin and trypsin were made.

Hunter and Smith[1] mentioned that erepsin was no more efficacious than trypsin in the production of ammonia from peptone. They show no data but state "unfortunately the erepsin preparations were not very active." Luck[5] showed that the trypsin-stable complex, which he isolated from a trypsin-casein digest, when incubated with pigs kidney-tissue extract liberated ammonia. This suggested the possibility of hastening the liberation of ammonia from a casein-trypsin digest by adding kidney-tissue extract.**

Experiment III. A casein-trypsin digest and casein control 30 days old were available. These were used as follows: 4 flasks, D and D′ each containing 100 cc. digest, and C and C′ each containing 100 cc. casein control. 50 cc. of kidney extract was added to D′ and C′; to D and C 50 cc. of distilled water was added.

It is evident from Table III that the kidney-extract was active;

TABLE III.

Time of Digestion	Casein Digest		Casein Control		Casein Digest +kidney-ext.		Casein Control +kidney-ext.*	
	mg. of N in 100 gm. of solution as				mg. of N in 100 gm. of solution as			
	NH_2	NH_3	NH_2	NH_3	NH_2	NH_3	NH_2	NH_3
	D		C		D′		C′	
0 hr.	332.94	45.92	81.16	11.9	332.90	45.94	81.16	11.92
1 day	332.90	47.81	83.2	12.1	593.7	52.92	194.1	20.2
5 days	333.0	49.00	88.0	14.0	621.0	55.44	319.9	26.04

* Correction for kidney-extract control was made.

[6] Cohen, E. J., *Physiol. Rev.*, 1925, **5**, 349.

** To 10 gm. fresh dog-kidney finely minced and ground with washed sand was added 50 cc. distilled water. The suspension was filtered through 2 layers of cheese-cloth. One part of extract was added to 2 parts of digest.

also, that the amidolysis as well as the proteolysis was greater in C′ than in D′ (greater concentration of "peptide substrate"). In no way, however, did these results show what caused amidolysis. They merely confirmed that ammonia was liberated along with amino nitrogen but at a much lower rate. The liberation of amino nitrogen was due to trypsins or erepsins.††

Experiment IV. Retarding effect of antiseptics. Lang[7] found that toluol is especially effective in retarding the formation of ammonia. To test the effect of tricresol or toluol (antiseptics used in these experiments) Experiment I was repeated with no preservative, *i. e.,* the protein solutions were prepared the same as in Experiment I but no toluol was added. The control as well as the solution to which the trypsin was added and thoroughly mixed were at once filtered aseptically through a sterile Berkefeld filter into sterile flasks and incubated at 37°C. Aliquot samples were taken for analysis, using bacteriological technique, sterile pipettes, etc., at time intervals as indicated. The results were identical with those of Experiment I. Thus, whatever the amidolytic activity in trypsin, it was not affected by the preservative used under the conditions of these experiments.

Summary. 1. In tryptic digestion the liberation of ammonia was due to an enzymic activity associated with the trypsin preparation used, whereas in peptic digestion the ammonia liberation was due to the acidity only. 2. The more rapid liberation at first in a pepsin digest than in the control can be explained on the basis of the physical and chemical change in the substrate due to peptic proteolysis. 3. Kidney-extract did not hasten ammonia liberation. The aminolytic and amidolytic activity in the extract was high and seemed to follow the same course as with trypsin. 4. The concentration of antiseptic used under the conditions described did not retard either activity.

The author wishes to express her appreciation to Professor F. C. Koch for his helpful criticisms and suggestions.

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†† It was shown in Experiment I that a further addition of Difco trypsin produced no further effect. Hence the action in the kidney-extract might have been due to a different trypsin or to some erepsin. Erepsin is said to be a mixture of enzymes which act on polypeptides and dipeptides. The action in this experiment is on casein. The addition of the kidney extract to a pepsin-HCl digest and control showed no activity, *i. e.,* all kidney-extract enzyme activity was destroyed at that pH.

[7] Lang, S., *Beitr. Z. Chem. Physiol.,* 1904, **51**, 321.

6526

Experimental Hypocalcemia.

A. B. HASTINGS AND C. B. HUGGINS.

From the Departments of Medicine and Surgery, and the Lasker Foundation for Medical Research, the University of Chicago.

Approximately 90% of the calcium may be adsorbed from the serum of normal dogs by shaking with solid $Pb_3(PO_4)_2$.[1] Using this method of decalcifying blood the following experiments have been carried out.

Small dogs were alternately bled maximally (about 50% of their blood volume) and immediately transfused with an equal volume of decalcified blood. This procedure was repeated as many as 28 times in the course of an afternoon. Each sample of blood removed was analyzed for calcium, phosphorus, and occasionally for CO_2 and pH. When the transfusions were repeated every 20 minutes the serum calcium did not decrease below 2.1 millimols per liter and no symptoms occurred.

When only 10 minutes were allowed to elapse between transfusions the serum calcium decreased to 1.6 millimols per liter after 12 or 13 transfusions. Mild symptoms of tetany appeared when the serum calcium reached 1.75 millimols per liter (7 mg. per 100 cc.). These persisted as long as the serum calcium was kept below this level. If an interval of 20 minutes or longer was allowed to elapse between transfusions the serum calcium rose promptly, even though the calcium removed had far exceeded that calculated as being initially present in solution in the body. This is interpreted as evidence that the bone salts are freely soluble in serum which has been rendered undersaturated with respect to them. Experiments of this nature were performed on 6 normal dogs. The cerebrospinal fluid was analyzed for its calcium content at the end of the experiment in one dog and was found to be 1.6 millimols per liter, a normal value.

The symptoms observed may be characterized as those of a mild tetany. At no time did a well-defined convulsion occur. Specifically the symptoms noted were localized but persistent tremors of groups of muscles of the extremities, chest, shoulders, and head. Sometimes involuntary movements of the fore legs occurred.

When the parathyroid glands were removed prior to the experiment, the following differences were noted. If the parathyroidectomy was performed 6 hours before the experiment, the initial serum calcium was normal. Transfusing with decalcified blood brought the serum calcium below the tetany level more rapidly than in the series of experiments on normal dogs, *i. e.,* within one hour instead of between 1¾ or 2 hours. The symptoms in these animals were mild, however, and the cerebro-spinal fluid calcium was not reduced appreciably below normal at the end of the experiment. Furthermore, in spite of 18 transfusions, extending over 3 hours, the blood was still able to take up calcium *in vivo* if the transfusions were interrupted.

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[1] Roseberry, H. H., and Hastings, A. B., unpublished results.

When the parathyroidectomy was performed 24 to 30 hours prior to the experiment, marked symptoms of tetany were brought on shortly after transfusions with decalcified blood were begun. The serum calcium could be kept at a lower level in such animals (1.3 millimols per liter). At the same time, the cerebro-spinal fluid calcium was found to be 1.4 millimols per liter.

6527

Effect of Calcium and Citrate Injections into Cerebrospinal Fluid.

C. B. HUGGINS and A. B. HASTINGS.

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Sabbatini observed that the direct application of isotonic solutions of oxalate or of citrate to the exposed brain of dogs increased their excitability and at times resulted in a generalized convulsion.[1] This effect was antagonized by calcium. We have repeated and extended these experiments. Small amounts (0.1-0.3 cc.) of isotonic solutions of either sodium citrate (0.077 M), calcium chloride (0.107 M), calcium citrate (0.06 M), or magnesium chloride (0.107 M) were injected into the cisterna magna of dogs, using a fine needle (26 gauge).

A typical syndrome occurred following injections of sodium citrate, consisting of immediate contraction of the voluntary muscles of the neck, trunk, extremities and tail. The motor phenomena included an extensor rigidity of the legs, slightly greater in the fore legs than in the hind, and a marked opisthotonos, pleurothotonos, and dorsal curvature of the tail. The heart rate slowed during this phase of the effect and was accelerated during the recovery period. Profuse salivation occurred. The intense motor excitation lasted usually from 5 to 15 minutes, but the animals were ataxic and had an unsteady wide-based gait for a somewhat longer period. In a minority of cases a generalized tonic and clonic convulsion followed the original motor spasm. The animals were usually normal 30 minutes after the injection.

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[1] Sabbatini, L., *Revista sperimentale di freniatria,* 1901, **27**, 946.

In some of the experiments, the needle was left in the cisterna following citrate injection and, as soon as the motor reaction was obtained, calcium chloride or magnesium chloride was injected in amounts equivalent to the sodium citrate. The hypertonic muscles relaxed in a few seconds and within a few minutes the animal appeared to be normal.

Injections of Ringer's solution and sodium chloride in these small doses were without apparent effect. Injection of calcium citrate did not provoke a motor response.

6528

Influence of Salt Intake on Susceptibility of Albino Rats to Diets of Low Vitamin B Content.

MARY C. PATRAS AND R. D. TEMPLETON. (Introduced by T. E. Boyd.)

From the Department of Physiology and Pharmacology, Loyola University School of Medicine.

Fifty-six rats were used in the studies here summarized. Before the experiment they had been kept on a stock diet of Purina Fox Chow, Miller's Puppy Meal, and oats fed daily, with the addition of meat, cheese, and cabbage once or twice a week. Litter mates of the same sex, and as nearly the same weight as possible, were selected. The animals were divided into 2 age groups, one varying from 25 to 55 days and the other from 91 to 148 days.

The controls, including half the rats in each age group, received diet No. 1, consisting of starch 64%, casein 18%, butter 10%, salts 3% (Osborne and Mendel's mixture as prepared by Harris Laboratories) and yeast 5% (Fleischmann's bakers' yeast, fresh). The yeast is at least 70% water, and the amount given is evidently inadequate for vitamin B requirement, since we have found that rats kept on this diet invariably die within a few weeks. The animals thrive on the diet if it is modified by trebling the yeast content. Diet No. 2, given to the remaining animals, was similar to No. 1 except that the salt mixture was entirely omitted and replaced by additional starch. The rats of both series received food and tap water in excess of the amount consumed.

During the first 14 days of the experiment the average weight was much greater for the control rats than for those on diet No. 2. The survival period, however, was longer for the latter group. By the 55th day all the mature controls were dead, while 85.7% of their litter mates on diet No. 2 were surviving, though with an average loss of 28.5 gm. from their initial weight. On the 58th day the last of the younger control rats died, while 58.1% of their litter mates on diet No. 2 were still living, with an average gain of 5 gm. over the initial weight. The weight curve for these younger rats was complicated by the growth factor and did not show the marked loss in weight exhibited by the mature animals.

The age of the rats within the range of 25 to 148 days did not seem to be an important factor in determining their resistance to vitamin B deficiency. The average initial gain in weight was somewhat greater for the young controls but their average duration of life was about the same as for the mature animals on the similar diet.

The average initial gain for the males of all ages studied was greater than the gain made by the females. The resistance to vitamin B deficiency, however, as measured by the duration of life did not seem to be greatly influenced by sex.

During the last few days of life, the control animals showed typical polyneuritic spasms which were frequently very severe. The animals receiving the salt deficient diet seldom showed even mild spasms. The majority of these rats died in coma.

The average survival period for all the controls was 46.5 days, while the animals receiving the salt deficient diet showed an average exceeding 77.2 days. On the 58th day when the last of the controls died, 67.8% of the rats on diet No. 2 were still living.

Shinza[1] reported that rabbits given an excessive amount of sodium salt developed a condition closely resembling experimental beri beri. He suggested that this might be due to a withdrawal of the potassium ion by the sodium. Whether our results can be explained on the basis of an imbalance between the potassium and sodium ions in the dietary salts remains to be studied.

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[1] Shinza, R., Scientific Rep. from the Govt. Inst. for Infect. Dis., The Tokyo Imp. Univ., 1923.

6529

A New Method for Production of Experimental Abscesses of the Lung in Dogs.*

W. M. TUTTLE. (Introduced by P. R. Cannon.)

From the Department of Pathology, University of Chicago.

The method devised by Holman,[1] Cutler,[2] Schleuter,[3] and Weidlein for the experimental production of lung abscesses has been used extensively with gratifying results by the originators. Van Allen and Adams,[4] and Tuttle and Nicoll,[5] have also demonstrated the reliability of the method.

The abscesses are produced by introducing an embolus composed of a small segment of the femoral vein of the dog into the external jugular vein of a dog and washed down with 0.85% NaCl. A small amount of bacterial suspension and a paraffined lead bird shot are put into the segment of vein and the ends tied with silk thread before the embolus is introduced.

These emboli, however, have certain disadvantages; for example, they do not all contain the same number of organisms, the vein wall is not absorbed after weeks or even months and the thread used to tie the ends and the lead shot constitute foreign bodies in the tissues. Furthermore, it is impossible to make microscopic sections of an abscess with the embolus *in situ* due to the thread and the lead shot.

In an attempt to eliminate these disadvantages, an agar embolus has been devised. The method is as follows: a bacto-beef agar is prepared, with an agar content of 5%. This is sterilized and cooled to 45°-50°C. and a known suspension of organisms is added, usually from 0.2 to 0.4 cc. of a 24-hour broth culture of *Staphylococcus aureus* or *B. coli*. The agar is then drawn into a sterile pipette, the lumen of which is the size desired for the embolus. The agar is allowed to harden and is blown from the pipette into a sterile petri dish. The agar is cut into 5-8 mm. lengths and the embolus is introduced into the external jugular vein of a dog and washed

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* This work was done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Holman, E., Weidlein, Schleuter, PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 266.

[2] Cutler, E. C., Schleuter, *Ann. Surg.*, 1926, **84**, 256.

[3] Schleuter, Weidlein, *Arch. Surg.*, 1927, **14**, 457.

[4] Van Allen, C. M., Adams, W. E., Hrdina, L. S., *Arch. Surg.*, 1929, **19**, 1262, 1279.

[5] Tuttle, W. M., Nicoll, G. L., *J. Thoracic Surg.*, 1932, **2**, 60.

down with 0.85% NaCl. If a radio-opaque embolus is desired lipiodol (5%) is added to the melted agar and emulsified. Such emboli more nearly simulate in form and consistency those forming from the blood constituents in man.

We have used the method thus far in 25 dogs and an abscess has developed in each instance. The abscesses so formed are true abscesses and not areas of coagulation necrosis. Microscopic sections are easily made with the embolus *in situ* and in quantitative work the presence of approximately the same number of organisms in each embolus is of advantage.

6530

Experimental Production of Bronchogenic Abscess of the Lung.*

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From the Departments of Surgery and Pathology, University of Chicago.

The etiology of post-operative lung abscess has been a subject of much interest and controversy during the past decade. Two theories have been presented as to the etiological factors of this condition, embolic and bronchogenic. Embolic lung abscess has been experimentally produced with almost routine regularity. Although most factors suggest a bronchial route of infection, little success has attended many ingenious methods by a large number of workers to produce this condition experimentally. The following method of producing bronchogenic lung abscess is presented because of its simplicity and dependability.

Eighteen dogs and 3 goats were narcotized with morphine sulphate. The infective organisms employed were human tubercle bacilli, H-119, which are a branch of the strain H-137. Under fluoroscopic control, a small ureteral catheter was passed through a bronchoscope into the finer ramifications of a tertiary bronchus of one of the lower lobes. By employment of the fluoroscope, the infective organisms could be placed in any desired part of the lobe. 0.2 cc. of a fairly heavy suspension of the organisms in lipiodol were injected into one or both of the lower lobes. The course of the lesion was followed by roentgen-ray. Cavities as large as 2.5 cm. in diameter could be demonstrated.

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Results. Some of the dogs died due to the magnitude of the abscess cavity or to the added burden of infection spread to adjacent lobes. Other dogs were sacrificed at intervals. There was practically a routine production of an abscess cavity from 1 to 2.5 cm. in diameter following the injection. One goat died following the administration of barbital. Another goat developed convulsions and died soon after operation. The third presented an abscess 2.5 cm. in diameter demonstrable by roentgen-ray.

Abscess cavities formed from 10 days to 2 weeks following injection, and persisted as long as 7 months. It might well be that a substance having a higher specific gravity would be necessary to retain infective organisms in a dog's lung than in a human lung, while an abscess was being produced. The above method is being used at present in an attempt to produce bronchogenic lung abscess with pyogenic organisms.

6531

Inverse Concentration Ratios for Sodium and Potassium in Gastric Juice and Blood Plasma.

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From the Department of Physiology, University of Illinois.

The acidity of the gastric juice is the result of the secretion of fluid at least as rich in chloride ion as the blood plasma but less concentrated with respect to fixed base. It has long been known that the sodium ion concentration in gastric juice is less than in blood plasma in direct ratio with the free acidity of the juice. The behavior of the other metallic cations has not been so carefully studied. Bliss[1] showed that the average potassium content of the gastric juice in the dog and man was consistently higher than the normal potassium concentration in the blood plasma. He presented no simultaneous analytical figures for blood and gastric juice in the same individuals, and consequently his conclusions could not be taken without qualification. Austin and Gammon[2] studied the potassium concentration in gastric juice but made no comparison with the concentration in the blood. We found it, therefore, of importance to study simultaneously the concentrations of sodium and potassium in the gastric juice in relation to their concentration in the blood at the time of secretion.

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[1] Bliss, T. L., *Ann. Int. Med.*, 1930, **3**, 838.

[2] Austin, J. H., and Gammon, G. D., *J. Clin. Invest.*, 1931, **10**, 287.

Dogs under ether or amytal anesthesia were used. The pylorus was cannulated and gastric juice collected after the injection of .03 mg. histamine in Ringer's fluid intravenously. Sodium was estimated by the Kolthof and Barber method[3] and potassium by the method of Breh and Gaebler.[4]

In 24 experiments in which the sodium and potassium concentrations in blood plasma and gastric juice have been studied we have found that the concentration of potassium is invariably greater in the gastric juice than in the plasma, and sometimes the former may be as much as 4 times the latter. On the other hand we have always observed, in conformity with previous workers, that the sodium concentration is markedly lower in gastric juice than in the plasma

TABLE I.
Ratio Between Concentration in Gastric Juice and in Blood Plasma for Potassium and Sodium.

		Potassium			Sodium	
	Blood	Gastric Juice	Ratio	Blood	Gastric Juice	Ratio
Exp.	mg./100 cc.	mg./100 cc.	G/B	mg./100 cc.	mg./100 cc.	G/B
1	16.41	39.98	2.43	355.5	253.1	.71
2	14.21	21.92	1.54	322.0	285.0	.88
3	7.56	18.36	2.42	336.8	281.9	.83
4	14.27	30.21	2.12	332.1	240.4	.72
5	16.26	20.75	1.28	337.1	230.2	.69
6	19.65	27.83	1.42	332.6	154.4	.43
7	11.81	28.34	2.40	330.8	111.5	.34
8	10.62	25.21	2.36	351.8	236.7	.67
9	9.67	33.32	3.45	318.8	135.9	.43
10	18.37	33.39	1.81	351.2	125.3	.35
11	24.06	89.00	3.70	360.3	271.3	.75
12	20.12	26.25	1.31	370.0	280.4	.76
13	14.29	39.84	2.72	347.3	269.7	.78
14	11.68	31.69	2.71	336.0	328.0	.98
15	7.26	18.59	2.56	334.1	234.4	.70
16	14.48	31.22	2.15	340.6	250.6	.74
17	16.00	17.59	1.06	348.3	244.6	.70
18	16.56	25.20	1.52	381.3	184.7	.49
19	9.18	22.41	2.44	356.0	190.8	.53
20	7.42	23.26	3.13	359.1	273.2	.76
21	8.09	30.21	3.74	358.6	248.8	.69
22	13.11	31.38	2.38	371.4	142.8	.38
23	18.51	61.60	3.32	392.4	218.3	.56
24	31.08	61.60	1.98	373.1	164.2	.44
		Mean	2.33			.64

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[3] Kolthof, I. M., and Barber, H. H., *J. Am. Chem. Soc.*, 1928, **50**, 1625.

[4] Breh, F., and Gaebler, H., *J. Biol. Chem.*, 1930, **87**, 81.

of the animal at the time of secretion. The results are shown in Table I.

It is rather surprising that the 2 monovalent cations, sodium and potassium, behave in this inverse manner with respect to their secretion in the gastric juice. In the ultrafiltration of plasma, as Ingraham, Lombard and Visscher[5] have shown, the potassium ion appears in the ultrafiltrate as a smaller fraction of its concentration in the plasma than does the sodium. Therefore, in any ultrafiltration process one would expect the potassium to be diluted to a greater extent than the sodium. Furthermore, in any distribution of ions set up by electrical potential differences operating to produce iontophoresis it would be expected that all cations would be concentrated or diluted in proportion to their migration velocities, but not that one cation species would behave qualitatively in a different manner from other ions of the same charge. Macallum[6] has pointed out that in mixtures of sodium and potassium salts potassium is selectively adsorbed to silica. He has suggested that the higher migration velocity of potassium ions may be responsible for this selective adsorption.

Tentatively we would suggest as the reason for the difference between the behavior of potassium and sodium in gastric juice that potassium enters the lumen of the gland from the secreting cells themselves, while the sodium reaches the lumen through the intercellular spaces. This is in accordance with the well-known fact that potassium occurs in high concentration within the cells, whereas sodium is very much less abundant. This difference is presumably due to differences in cell membrane permeability with respect to ions as well as to the specific adsorption effect noted by Macallum.

6532

A Simple Method for Staining Fungi.

EMIL WEISS. (Introduced by Lloyd Arnold.)

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The ordinary dyes, when applied to bacteria, do not stain well the mycelium and the spores of the fungi. Slightly better results are obtained if the dyes are steamed. Some procedures apply Gram's iodine, after the respective dye was used. Similarly, the use of spore staining methods is less satisfactory as compared with those observed in staining bacterial spores. In order to obtain more effective staining, the influence of mordants was tested. Smears of fungi were steamed with a mordant preceding the staining. This mordant consists of 2 solutions: Solution A., contains 100 gm. of tannic acid dissolved in 100 cc. of 95% alcohol, and solution B., which consists of 92.5 cc. of 50% formalin, and 7.5 cc. of glacial acetic acid. Before use, one part of solution A and 2 parts of solution B are mixed uniformly; this mixture represents the mordant to be used. Separately, either solution keeps indefinitely. The fixed smear is covered with the mordant-mixture, steamed for 5 minutes, and washed off the slide with warm water. The slide is covered with the respective stain, steamed for 3 to 5 minutes, and washed.

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[5] Ingraham, Lombard and Visscher, *J. Gen. Physiol.*, in press.

[6] Macallum, A. B., *Proc. Roy. Soc. London*, Sect. B, 1928-29, **104**, 440.

Used after the mordant, almost any of the ordinary dyes give satisfactory results. Particularly good results were obtained with Loeffler's methylene blue, gentian-violet, brilliant green, fuchsin, and safranin. In instances where darker staining is required (for photographic purposes), the application of the stain is followed by steaming with Gram's iodine for 3 to 5 minutes, thus rendering the fungi and their spores almost black (if methylene blue or gentian violet is used).

The application of the mordant also greatly improves the contrasting spore staining methods of bacteria when applied to fungi. The procedure is as follows: The fixed smears are steamed with the above mordant for 5 minutes as above, and washed off with warm water. Methylene blue, or brilliant green is added and steamed for 3 to 5 minutes; the slide is thoroughly washed with water, and 1% acid-alcohol applied until most of the dye appears removed. Ordinary fuchsin or safranin is used for one-half minute, or until the smear assumes a reddish color. The excess dye is removed by washing with running water. The slide is dried and examined. The mycelium appears distinctly red stained, while the spores, regardless of their location or arrangement, assume a deep blue or green color.

6533

Experimental Jejunal Ulcer: Relative Importance of Mechanical and Chemical Factors.

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From the Seymour Coman Fellowship and the Douglas Smith Foundation, Departments of Physiology and Medicine, University of Chicago.

The purpose of this work was to study the respective rôles of "acid neutralization" and trauma in the pathogenesis of the experimental ulcers produced in dogs by the duodenal drainage operation of Mann and Williamson.[1] Since the inception of our studies other investigators, notably Beaver and Mann,[3] Morton,[2] Matthews and Dragstedt,[4] have made significant contributions to the problem, which are quite in accord with our observations.

Methods. The technique described by Mann and Williamson and by Morton was followed. The operation consisted of dividing the stomach and duodenum at the pylorus, turning in the blind end of the duodenum, dividing the duodenum and jejunum at approximately the duodenal-jejunal flexure, joining the stomach to the divided end of the jejunum by an end-to-end anastomosis, and attaching the distal end of the duodenum to the small bowel by a side-to-side or an end-to-side anastomosis. The point of this union, that is, the site of duodenal drainage, was varied, being near the gastro-enterostomy stoma in some and the ileo-cecal valve in others. In the first series, all other factors were kept constant, except for some variation in the size of the gastro-enterostomy stoma resulting from differences in the size and age of the dogs. The purpose of these experiments was to study the importance of the site of duodenal drainage, *i. e.,* high or low in the intestine, in the incidence and rapidity of ulcer formation.

The second series was designed to study the rôle of trauma. In addition to carrying out the same procedures as in the first group, traumatic lesions were produced within the first few centimeters of the jejunum, just distal to the gastro-enterostomy, by the application of a clamp in the manner described by one of us (W.J.G.)[5] In normal animals such lesions heal in from 15 to 31 days.

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[1] Mann, F. C., and Williamson, C. S., *Ann. Surg.*, 1923, **77**, 409.

[2] Morton, Charles Bruce, *Ann. Surg.*, 1927, **85**, 207.

[3] Beaver, Meredith G., and Mann, F. C., *Ann. Surg.*, 1931, **94**, 1116.

[4] Matthews, Warren B., and Dragstedt, Lester R., "Experimental Studies of Gastric and Duodenal Ulcer." *Surg., Gynecol., Obstet.*, in press.

[5] Gallagher, William J., *Arch. Surg.*, **15**, 689.

Results. Table I shows a summary of the results obtained in the first group. Typical jejunal ulcers developed in 17 of the 22 animals, an incidence of 77.3%. This is somewhat lower than that obtained by Mann and Williamson and by other workers. Death from perforation of the ulcer and subsequent peritonitis occurred in 5 animals. Marked inanition was present in 14 dogs, and seemed to produce death. In 11 of these, ulcers were present, but in 3 others, death resulted from inanition without ulcer formation. In these, the anastomosis was high, and the cause of inanition was not apparent. After the operation an initial loss of weight occurred which was followed by a period of maintenance or even of gain in weight. With proper care the animals seemed to do quite well for an indefinite period. With the appearance of loss of appetite and rapid loss of weight, ulcer formation was suspected and usually confirmed at autopsy within a few weeks.

TABLE I.
The Effect of the Site of Drainage on Ulcer Formation

Days Duration	Circumference Stoma cm.	Dist. from Site of Drainage to		Ulcer Formation	Cause of Death	Remarks
		Stoma cm.	Ileo-cecal Valve cm.			
100	—	59	140	0	Inanition	Distemper and Acute Parotitis
182	4.0	68	242	Present	,,	
619	—	Lower Ileum	—	,,	Sacrificed	Lost wt. rapidly last 4 mos.
187	4.0	62	170	,,	Inanition	
354	4.2	82	216	,,	Hemorrhage and Inanition	
357	3.5	123	283	0	Distemper	
210	4.0	80	225	Present	Peritonitis from perforated ulcer	
130	—	82	212	,,	,, ,,	
351	3.5	145	220	,,	Inanition	
59	—	—	—	,,	,,	
89	4.0	280	186	,,	,,	
126	4.0	54	123	,,	Peritonitis from perforated ulcer	
35	6.0	250	50	,,	Inanition	
29	4.5	180	50	,,	,,	
30	3.5	203	56	,,	Peritonitis from perforated ulcer	
23	4.0	217	62	0	Distemper	
187	—	12	228	Present	Peritonitis from perforated ulcer	
289	3.0	9.5	214	0	Inanition	
169	—	12	—	0	,,	
150	—	12	—	Present	,,	
356	4.1	16	258	,,	Hemorrhage and Inanition	
54	3.0	12	—	,,	,, ,,	

Total No. 22. Incidence of ulcer formation 77.3%.

The site of the drainage did not influence the frequency or rapidity of ulcer formation. In dogs 163, 172, 175, 179, and 196 the site of drainage was high; the animals lived 187, 289, 169, 150, and 356 days respectively. In dogs 6, 28, and 34, on the other hand, the drainage was low and yet the animals lived 619, 351, and 89 days respectively. The great variability in ulcer formation is illustrated by the time required for the dogs with very high anastomoses, that is, in which the point of drainage was placed within 16 cm. of the

TABLE II.
Rôle of Operative Trauma in Ulcer Formation.

Days Duration	Circumference Stoma cm.	Dist. from Site of Drainage		Ulcer Formation	Traumatic Lesions	Cause of Death
		to Stoma cm.	to Ileo-cecal Valve, cm.			
36	—	170	95	Present	Healed	Hemorrhage, Inanition
48	2.6	117	70	0	”	”
35	3.5	280	35	Present	”	Peritonitis from perforated ulcer
22	4.0	115	50	0	”	Inanition
*59	4.0	166	56	Present	Chr. Ulc.	Peritonitis from perforated ulcer.
71	5.0	165	34	”	Healed	Inanition
14	4.0	190	50	0	”	Distemper
65	3.0	120	50	Present	”	Inanition
31	3.5	170	47	0	”	”
23	3.5	170	80	Present	”	Peritonitis from perforated ulcer
26	—	—	50	0	”	Intussusception of ileum
57	3.5	220	56	Present	”	Peritonitis from perforated ulcer
78	4.0	160	105	”	”	Inanition
45	3.0	110	40	”	”	Peritonitis from perforated ulcer
57	2.5	170	57	”	”	” ”
*19	3.5	220	75	”	Unhealed	” ”
59	3.2	135	80	0	Healed	Foreign body (sponge) abscess
*39	3.0	170	100	Present	Chr. Ulc.	Peritonitis from perforated ulcer
41	3.0	210	110	”	Practically healed	” ”
26	3.5	7.0	—	0	Healed	Sacrificed
147	2.5	28	217	Present	”	Peritonitis from perforated ulcer
94	4.0	5.0	—	”	”	Inanition
33	2.5	7.0	—	0	”	”
33	3.5	6.5	—	0	”	”
88	3.0	7.0	210	Present	”	Peritonitis from perforated ulcer

Total number 25. Incidence of ulcer formation, 64% (16 cases).
Incidence of unhealed traumatic lesions, 12% (3 cases).

*The possibility cannot be excluded that these are instances of spontaneous ulcer formation occurring at the site of the trauma, but independent of it.

gastro-enterostomy stoma, to develop ulcers and die: 187, 289, 169, 150, 356, 54, 94, and 88 days.

Table II shows the group in which traumatic clamp lesions were also produced. The incidence of spontaneous ulcer formation was somewhat lower than in the first group, being 64%. The traumatic lesions apparently healed easily even though spontaneous ulcers developed near by. In 3 instances the traumatic lesions apparently failed to heal. It must be admitted that even in these 3 a spontaneous ulcer might have formed at the same site if trauma had not been applied. In this group as in the first, no direct relationship was noted between the incidence and rate of ulcer formation and the distance between the stoma and the point of duodenal drainage.

Ulcer formation was noted in 33, or 70.2% of the total of 47 cases in the 2 groups. The incidence of death from peritonitis secondary to perforation of the ulcer was 34% (16 cases).

Many of the lesions were solitary (Fig. 1), but frequently they were multiple. In one instance (Fig. 2), 8 discrete ulcers were seen. The lesion nearest the stoma was invariably the largest. The site of predilection was the anterior superior wall of the jejunum, 0.5 to 1.0 cm. distal to the suture line. Occasionally the ulcer extended back to the suture line. The lesions varied in size, usually

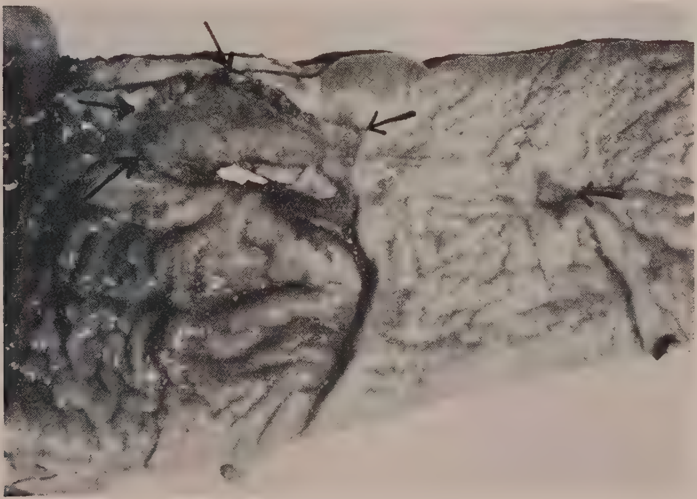

FIG. 1.

Dog No. 111. Duration of life 39 days. Death due to peritonitis from perforated ulcer. The smaller lesion is located at the site of clamp trauma.

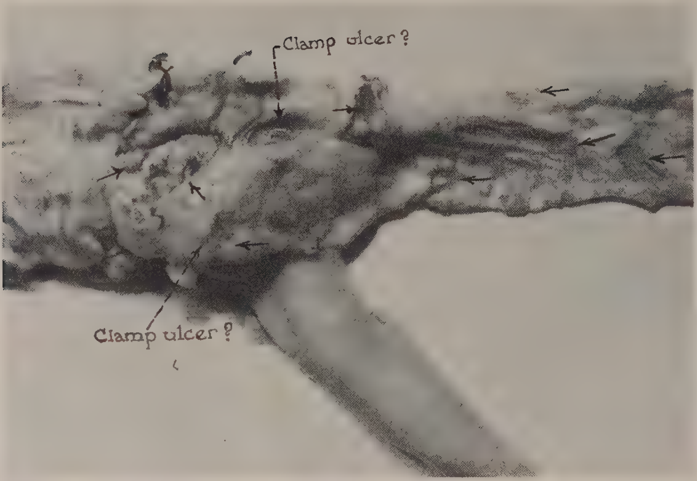

FIG. 2.

Dog. No. 109. Duration of life 19 days. Death due to peritonitis from perforated ulcer. Photograph shows 8 discrete ulcers, 2 occurring at the approximate site of clamp trauma.

being from 1 to 3 cm. in diameter and 0.5 to 1.0 cm. in depth. They were crater-like, indurated, with precipitous or sloping margins.

The time required for death due to ulcer formation ranged from a minimum of 19 days to a maximum of 356 days. In one experiment the animal was sacrificed after 619 days, an ulcer being found at autopsy. The explanation of this variability is not apparent.

No relationship seemed to exist between the size of the stoma and the rapidity with which the lesion developed. This has been confirmed by Jenkins,[6] who has regularly found ulcers in such animals even though a wide side-to-side gastro-enterostomy stoma is employed.

Studies were made of the gastric acidity following operation, the test stimulus used being a milligram of histamine injected subcutaneously. Free acidity was invariably present, the maximum concentration of hydrochloric acid as determined by titration usually being between 0.2% and 0.4%.

This work is further confirmation of the original observations of Mann and Williamson, although the incidence of ulcer formation was slightly less than in their series. The site of duodenal drainage, *i. e.*, whether high or low, seems to be of relatively little importance.

The regularity with which the traumatic lesions heal even though new ulcers form seems to us to definitely exclude operative trauma as a significant factor in the pathogenesis of the ulcers. It is difficult to understand, however, how a chemical factor alone can be the effective agent. The clamp lesions heal in the same areas in which new ulcers form, bathed by the same fluid. An attractive hypothesis is suggested by the work of Whitlow, who found that the gastric mucosa bleeds readily when the protective layer of mucus is wiped away and hydrochloric acid or acid gastric juice is then applied to the unprotected surface. It may be that the continued ejection of acid gastric chyme is similarly effective in removing the protective layer of mucus from a certain small area of the jejunal mucosa and in traumatizing the cells, thus rendering them susceptible to the attack of chemical agents.

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[6] Jenkins, H. P., to be published.

Conclusions. 1. In a total series of 47 animals, the duodenal drainage operation of Mann and Williamson resulted in jejunal ulcer formation in 38 instances, 70.2%. 2. The incidence of death from peritonitis secondary to perforation was 34%. 3. There was no relationship between the site of drainage, *i. e.*, whether high or low in the intestine, and the incidence or rapidity of ulcer formation. 4. Traumatic lesions of the jejunum healed promptly in spite of the formation of new ulcers in adjacent areas. 5. The experimental ulcers under consideration are not due to operative trauma, or merely to the chemical digestive action of the gastric juice, or to the two together. It is quite possible, however, that the continued mechanical and chemical trauma inflicted on the cells of the jejunal mucosa by the ejected acid chyme may be entirely responsible for the formation of the lesions.

6534

Bromide and Chloride Distribution Between Serum and Cerebrospinal Fluid.

M. MISHKIS, E. B. RITCHIE AND A. B. HASTINGS.

From the Lasker Foundation for Medical Research and the Department of Medicine, University of Chicago.

The report that bromide, after administration by mouth, exists in a higher concentration in the blood serum than in the cerebrospinal fluid attracted our attention because of the following considerations. If equilibrium exists between serum and cerebrospinal fluid, if bromide ions are freely diffusible, and if the protein ions are the only restraining factor, the application of the Gibbs-Donnan Law would lead to a predicted distribution ratio of $(Br)_s/(Br)_{csf} = 0.95$.

The bromide distribution ratios of Malamud, Fuchs and Malamud[1] were approximately 3.0 for normal individuals and did not fall below unity in patients with damaged permeability, as in paresis. Since the colorimetric method employed by these investigators did not permit the determination of actual bromide concentrations, observations have been made using the electrometric titration method previously described[2] as well as the colorimetric gold chloride method. The procedure followed was to administer to the patients 0.01 gm. of sodium bromide per pound of body weight 3 times a day for 5 days. Twenty hours following the last dose of bromide, blood and cerebrospinal fluid were drawn for analysis. This procedure was followed with 5 patients. Two others, O and R, were given larger doses of sodium bromide for a longer period before analyses were made. The results of 3 paretic and 2 non-paretic patients are given in Table I. Concentrations have been expressed in millimols per kilogram of water.

In all instances, with both methods, the ratio $(Br)_s:(Br)_{csf}$ was greater than unity. This substantiates the theory that bromides are not freely diffusible between serum and cerebrospinal fluid. In all except one instance the bromide ratio determined electrometrically was lower than that obtained colorimetrically.

TABLE I.
Halide Distribution Between Blood Serum and Cerebrospinal Fluid Following Sodium Bromide Administration.

	Electrometric Method									Colorimetric Method	
Subject	$(Br+Cl)_s$	$(Br+Cl)_{csf}$	$(Br)_s$	$(Br)_{csf}$	$(Cl)_s$	$(Cl)_{csf}$	$\frac{(Br+Cl)_s}{(Br+Cl)_{csf}}$	$\frac{(Cl)_s}{(Cl)_{csf}}$	$\frac{(Br)_s}{(Br)_{csf}}$	$\frac{(Br)_s}{(Br)_{csf}}$	Diagnosis
Li	—	—	—	—	—	—	—	—	—	2.86	Paresis
Li	111.5	128.3	4.8	3.4	106.7						”
S	111.0	134.5	8.4	2.4	102.6	132.1	0.83	0.78	3.50	2.72	”
Le	111.8	127.2	6.4	3.2	105.4	124.0	0.88	0.85	2.00	2.50	”
F	111.8	126.2	6.5	3.3	105.3	122.9	0.89	0.86	1.97	3.1	Eczema
G	—	—	5.2	3.3	—	—	—	—	1.57	2.80	Luetic Aortitis
O	107.4	124.0	36.4	19.0	71.0	105.0	0.87	0.68	1.92	—	Duodenal Ulcer
R	—		24.8	12.1	—	—	—	—	2.05	—	Epilepsy

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[1] Malamud, W., Fuchs, D. M., and Malamud, N., *Arch. Neur. and Psych.*, 1928, **20**, 780.

[2] Hastings, A. B., and Van Dyke, H. B., *J. Biol. Chem.*, 1931, **92**, 13.

Although our series is too short to be conclusive, there is no evidence of a difference in bromide distribution in paretic and non-paretic individuals. The total halide distribution ratio $(Br+Cl)_s$: $(Br+Cl)_{csf}$ was practically constant, 0.85-0.89 in the 5 cases in which it was determined. It should be noted that this is lower than that observed for the system, serum : edema fluid, and that predicted for the system by the Gibbs-Donnan distribution law. This agrees with the values for the ratio $(Cl)_s$:$(Cl)_{csf}$ found in the absence of bromide by others,[3, 4]

6535

The Effect of High Doses of Irradiated and Non-Irradiated Ergosterol on the Albino Rat.*

J. T. HAUCH. (Introduced by F. C. Koch.)

From the Department of Physiological Chemistry, University of Chicago.

There have been many conflicting reports concerning the toxicity of irradiated products. This report covers the work of 4 years throughout which we endeavored to follow procedures which closely resemble the clinical administration of these irradiated products. We used a commercial product prepared and furnished to us by Mead Johnson and Company.

All materials were reassayed by us for vitamin D potency by the McCollum line-test. We also studied the blood calcium and phosphorus of representative animals at various stages. Our high dosages of irradiated ergosterol varied from 100 X to 465,000 X the therapeutic dose. All animals were carefully weighed and observed for their behavior. A record of the matings and condition of all litters was kept. All animals sacrificed or found dead were carefully autopsied and microscopic sections made of the thyroid, thymus, aorta, heart, lungs, liver, stomach, duodenum, spleen, kidneys, and gonads.

In summarizing our results we can say that: 1. Special, very potent commercial preparations of irradiated ergosterol were stan-

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[3] Fremont-Smith, F., Dailey, M. E., Merritt, H. H., Carroll, M. P., Thomas, G. W., *Arch. Neur. and Psych.*, 1931, **25**, 1271.

[4] Muntrogler, E., Way, C. T., Pomerene, E., *J. Biol. Chem.*, 1931, **92**, 733.

* This investigation was supported in part by fellowship funds from Mead Johnson and Company.

dardized by the McCollum line-test and found to possess the claimed activity. 2. Ten adult albino rats on stock diet plus 100 to 800 X the therapeutic dose for 36 weeks were without observable harmful effects. 3. The progeny, consisting of 48 second, 35 third, 4 fourth, 18 fifth, and 12 sixth generation rats, were placed on the same stock diet plus doses up to 50,000 X the curative dose without observing harmful effects. The sixth generation animals were treated for 18 weeks, all the others for longer periods. 4. Pure ergosterol was irradiated in the solid form by a Cooper-Hewitt mercury arc lamp and fed to albino rats and their offspring in amounts up to 25,000 X the curative dose for 10 to 23 weeks without harmful effects. 5. Albino rats were placed on the McCollum 3143 diet with and without the commercial preparation in amounts up to 10,000 X the therapeutic dose. Although most of the animals died in less than 30 weeks, no true hypervitaminosis could be detected. 6. Young albino rats on a stock diet including bread and milk plus 93,000 X the curative dose for 12 months showed a loss of weight in some cases. Adult animals under the same conditions showed an immediate loss of weight and in some cases died with pathological findings similar to those indicated below. 7. Young and adult albino rats on a stock diet including bread and milk plus 465,000 X the therapeutic dose showed immediate toxic effects. These were immediate loss of appetite and weight, bloody discharge from the nose, diarrhea, marked muscular flabbiness, and gross pathological changes involving enlarged adrenals, atrophied thymus, and abnormal appearance of the heart and kidneys. The adult animals were again more susceptible than the young ones. 8. Rachitic diet was substituted for the stock diet on the 46,500, 93,000, and 465,000 dosage experiments on young animals. This change made the animals more susceptible. 9. Potassium iodide was added to the dosage of 93,000 X the curative amount without changing the picture. 10. Young from mothers on 10,000 X the therapeutic dose were weaned and placed on a rachitic diet. Only very slight rickets developed. Similar young nursed by stock diet foster mothers developed typical rickets. 11. These results indicate that the toxic effects observed by others on relatively low dosages are due to the presence of a toxic substance other than the true antirachitic agent in appreciable amounts in the preparation tested. Whether the toxic effects observed by us on our commercial preparation when given in very high doses are due to a trace of this hypothetical by-product or to the vitamin D substitute remains to be determined. 12. Our results when compared with others again indicate that the toxicity of a given irradiated ergosterol preparation is also determined in part by the character of the diet. In general the more complete and better balanced diets, act in a more protective manner.

Pacific Coast Section.

University of California Medical School, December 14, 1932.

6536

Dilatation of the Spleen. Stimulation of Nerves; Effect of Autonomic Drugs.

PAULINE HODGSON AND J. M. D. OLMSTED.

From the Division of Physiology, University of California.

Schafer and Moore,[1] using dogs as experimental animals and measuring splenic volume by use of the plethysmograph, obtained in only 2 isolated cases dilatation of the spleen as a result of stimulation; the first by slow and weak stimulation of the splanchnic nerve, the second by stimulation of the splenic nerve of an excised, perfused spleen 3 hours after removal from the body. Cleland and Tait,[2] also using dogs and measuring the length of the exteriorized spleen, obtained dilatation of the spleen only as a result of constriction of the splenic vein when they stimulated the vein directly, or the phrenic nerve by which this vein is innervated.

In the majority of our experiments we used decapitate cats, but in several cases we employed amytalized dogs and cats. Since plethysmographic methods for registering volume of the spleen, particularly increases in volume, were found to be unsatisfactory, changes in the area of the exteriorized spleen, registered by the method of Cook and Rose,[3] were taken as a measure of volume changes.

We found that, independent of blood-pressure changes, stimulation of the splanchnics on either side, or of branches of the splenic nerve craniad or caudad to the splenic artery, by means of a tetanizing current of weak intensity, produced dilation of the spleen in both the cat and dog, an increase of about 12% of its resting value. Slow break shocks of different intensities all proved to be ineffective. Any form of stimulation of the abdominal vagus was without effect, nor were we able to obtain dilatation of the spleen by stimulation of the splenic vein with a weak tetanizing current. These experiments indicate the presence of dilator fibers for the spleen running in the splanchnic nerves.

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[1] Schafer, E. A., and Moore, B., *J. Physiol.*, 1896, **20**, 1.

[2] Cleland, J. G. P., and Tait, J., *Quar. J. Exp. Physiol.*, 1927, **17**, 179.

[3] Cook, S. F., and Rose, M. I., *Am. J. Physiol.*, 1930, **92**, 240.

Effect of Autonomic Drugs. Classification of the autonomic nervous system into sympathetic and parasympathetic divisions has been based in part upon the responses of various tissues to certain drugs. A study of the responses of the spleen to the intravenous injection of the so-called autonomic drugs and to stimulation following such injection have given evidence for the nature of the innervation of the dilator mechanism.

Decapitate cats, or excised perfused spleens in a few cases, were used and the drugs were chosen in accordance with their generally accepted relations to the autonomic system as follows: adrenalin, motor to the sympathetic; ergotoxin and ergotamin, inhibitory to the motor sympathetic; pilocarpin, physostygmin, and acetyl cholin, motor to parasympathetic; and atropin, inhibitory to parasympathetic (including or excepting the dilator fibers). The dosages used were those recommended by Sollman,[4] for vascular and systemic effects. It is recognized that, with the possible exception of adrenalin, none of these drugs is strictly confined to either sympatho-mimetic or parasympatho-mimetic action, and that interpretation of results obtained must be made with caution.

Dilatation of the spleen was produced by, (a) the injection of pilocarpin, following atropin or ergotoxin when the adrenals had been ligated, (b) the injection of a small dose of acetyl cholin into the decapitate animal or into the perfusing fluid of the excised spleen, and (c) the stimulation of the splanchnic or the splenic nerves with a weak tetanizing current after the injection of atropin, ergotoxin or ergotamin.

There was no effect upon the spleen, when (a) adrenalin was injected following ergotoxin, or (b) pilocarpin or physostygmin was injected into the perfusing fluid of the excised spleen.

As evidence for the sympathetic and against the parasympathetic innervation of the dilator mechanism may be offered: (a) the dilatation produced by weak stimulation after atropin injection, (b) the dilatation following pilocarpin injection after atropin in the decapitate cat, and (c) the failure to produce dilatation where pilocarpin or physostygmin was added to the perfusate. This interpretation, in the first two cases, is based upon acceptance of the theory that atropin paralyzes all parasympathetics and that pilocarpin may act upon the sympathetic.

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[4] Sollman, T., A Laboratory Guide in Pharmacology, Philadelphia, W. B. Saunders Co., 1917.

As evidence for the parasympathetic control may be offered; (a) the dilatation following addition of small doses of acetyl cholin to the perfusing fluid of the excised spleen, (b) the dilatation produced by acetyl cholin or pilocarpin after ergotoxin into the decapitate cat, and (c) the dilatation produced by weak stimulation of the splanchnics or injection of pilocarpin after atropin, this interpretation being based upon the assumption that parasympathetic dilators are not paralyzed by atropin.

Evidence against the sympathetic control is offered by the fact that adrenalin did not produce dilatation of the spleen, after ergotoxin, even though reversal of the usual systemic effects of adrenalin was obtained. Since the action of adrenalin parallels so closely the action of the sympathetic system, the hypothesis that the dilators of the spleen are of parasympathetic origin is strongly supported by the failure of adrenalin to produce dilatation under these circumstances.

Probable course of dilator fibers through the ventral spinal roots. Kuré and his associates,[5] have concluded from histological and physiological evidence that vasodilator fibers to certain viscera are carried in the splanchnic nerves, that they come from the cord *via* the dorsal roots, are truly efferent fibers and are all of parasympathetic origin.

We were unable to obtain dilatation of the spleen in either the cat or the dog by weak stimulation of the dorsal roots of the 11th, 12th, or 13th post-cervical nerves, or of the cord, when the ventral roots had been sectioned. Weak stimulation of the ventral roots of these same spinal nerves or of the cord, when the dorsal roots had been sectioned, produced dilatation of the spleen in several preparations.

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[5] Kuré, K., *et al.*, *Quar. J. Exp. Physiol.*, 1928, **18**, 333; 1930, **20**, 51; 1931, **21**, 1, 103, 119.

6537

Spontaneous Psittacosis Infections of the Canary and Butterfly Finch.

K. F. MEYER AND B. EDDIE.

From the California State Department of Public Health and the George Williams Hooper Foundation, University of California.

The epidemiologic reports by Roubakine,[1] Sturdee and Scott[2] and Armstrong[3] mention instances of human psittacosis cases associated with canaries. Furthermore, Elkeles,[4] Levinthal[5] and Blanquinque[1] have experimentally proven the susceptibility of canaries and finches to the virus. An opportunity was afforded to study a group of canaries which were associated with 2 human cases for the presence of the virus, and thus to establish conclusively the spontaneous existence of psittacosis in this species.

Late in March, 1932, Dr. James B. Luckie reported 2 cases of psittacosis—a man and his wife whose only contact was with canary birds (*Serinus canaria*). About 2 weeks before the onset of the illness of Mrs. H. a canary had been bought from a pet shop in South Pasadena. This bird was placed in a cage with 12 other canaries. It died within a few days and the carcass was destroyed. The 12 canaries which had been in contact with the sick bird, were chloroformed about 6 weeks after the introduction of the sick bird. At autopsy the spleens of 2 birds were slightly elongated and thickened (9 and 11 mm. long) and the livers were mottled, fatty and enlarged. In the remaining 10 canaries the sizes of the spleens varied from 6 to 10 mm. The livers and spleens were thoroughly macerated in broth (10% suspension) and injected intraperitoneally into mice. A relatively weak virus was demonstrated in the viscera of 3 canaries. It is of interest that one of the Serinuses revealed no anatomical markings suggestive of infection. The virus, apparently present in small amounts, was readily passaged. This is evidenced by the schematic presentation of the investigation of canary 192 in Chart 1.

While inspecting an aviary a listless butterfly finch or nonpareil

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[1] Roubakine, *Monthly Epidemiological Report, League of Nations*, 1930, **4**, 170.

[2] Sturdee and Scott, *Rep. on Pub. Health and Med. Subjects*, London, 1930, **61**, 19.

[3] Armstrong, *Pub. Health Rep.*, 1930, **45**, 2014.

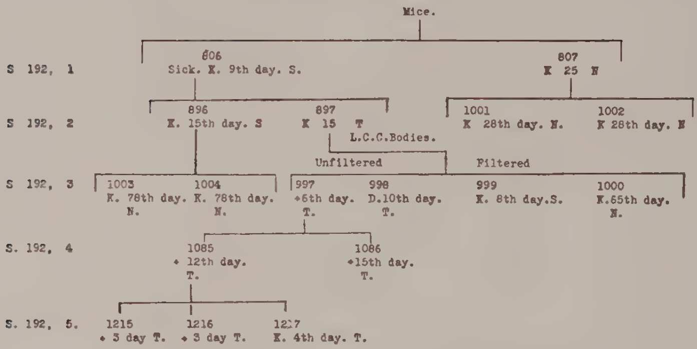
[4] Elkeles, *München. med. Wchnschr.*, 1930, **77**, 139.

[5] Levinthal, *Klin. Wchnschr.*, 1930, **9**, 654.

SERIAL PASSAGE OF SPLEEN AND LIVER OF CANARY 192

SPLEEN: 0.6 CM. LONG+ THICK

LIVER: SOFT AND FATTY



+ = died, K = killed, T = typical lesions, S = enlarged spleen, ballooned duodenum, but no necroses, N = normal viscera.

(*Cyanospiza ciris*) with ruffled plumage in a pen with infected parrakeets attracted attention. A close examination showed soiled tail feathers and a distended abdomen. The autopsy of this slightly emaciated male bird revealed an enlarged mottled liver of deep saffron yellow, an enlarged spleen, liquid intestinal content and empty crop and gizzard. Culturally the organs were sterile and L. C. L. bodies were not found. Suspensions of the ground liver and spleen killed mice in 10 days with lesions and elementary bodies typical of the psittacosis virus. Successive passage through mice shortened the incubation time and intensified the anatomical lesions.

In view of the above findings it is apparent that birds other than the representatives of the psittacine family, may be afflicted with a disease that is highly communicable to man. The presence of such an infection is easily demonstrated by mouse inoculation tests.

6538

Latent Psittacosis Infections in Mice.

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Krumweide and his coworkers,[1] Rivers and Berry,[2] and Bedson[3] have shown that mice are readily infected by intraperitoneal inoculations of the virus of psittacosis derived from birds or from man. Not only is the disease in mice less dangerous to the laboratory workers but the animal is inexpensive and reacts fairly uniformly to the virus. On account of the existence of latent infections parrakeets cannot be used for diagnostic work. The Japanese ricebird is quite expensive although excellent for the study of the elementary bodies and other related problems. In the course of an epidemiologic investigation of psittacosis in aviaries and in connection with diagnostic studies on human infections over 3,500 mice have been injected intraperitoneally with organ suspensions, blood or sputum specimens. The presence of a potent virus is indicated by illness and by typical anatomical lesions and death of the animals in from 4 to 30 days. However, a number of mice may show no signs of disease and yet harbor the virus in the spleen and liver many months after the inoculation of the suspected tissue emulsion or sputum. The following observations selected at random may illustrate this statement.

On January 12th 2 ricebirds and 4 mice were inoculated with emulsions of the spleen and liver of a dead parrakeet which exhibited the anatomical lesions of psittacosis. The ricebirds died on the 7th day with typical findings. The 4 mice remained well and when sacrificed on the 132nd day presented enlarged spleens. Subinoculations into mice caused fatal infections on the 9th and 10th day. The virus was easily propagated in series.

Mice injected with the enlarged spleens or nasal mucosa of several parrakeets failed to sicken. At autopsy 88, 57, 53 and 44 days respectively after the inoculation the spleens were found to be enlarged and by passage the virus of psittacosis was demonstrated. Three of 4 mice injected with the washed sputum of a contact case

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[1] Krumweide, *Science*, 1930, **71**, 262.

[2] Rivers and Berry, *J. Exp. Med.*, 1931, **54**, 105.

[3] Bedson, *Rep. on Pub. Health and Med. Subjects*, London, 1930, **61**, 85.

(nurse to case T) succumbed to a streptococcic septicemia. The remaining animal of the series was sacrificed on the 41st day and again subinoculations yielded the psittacosis virus. In the meantime a second specimen of sputum from the same patient had produced typical infections in 4 mice.

These and many similar observations confirm the fact that the psittacosis virus has a remarkable tendency to remain latent in the spleen and liver of birds and mammals. The subclinical disease is largely influenced by the amount and the potency of the virus. Latent infections occur more frequently in heterogeneous transmissions, for example, from birds to mammals, than when passages between mammals of the same species are attempted. From a practical diagnostic point of view it is important to reinoculate the spleens which are enlarged. This procedure renders the search for the psittacosis virus slightly more complicated but improves the accuracy of the mouse test.

Mice with latent infections resist reinoculations with a very potent virus. This resistance is sometimes followed by a sterile immunity. But since the time of autosterilization is uncertain the immunization with formalinized spleen emulsions is generally preferred.

6539

Latent Psittacosis Infections in Shell Parrakeets.

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The existence of the psittacosis virus in the budgerigar or shell parrakeet (*Melopsittacus undulatus*) bred and raised in California was demonstrated by laboratory tests. Forty-five human cases of psittacosis infection recorded in California have followed exposure of patients to this species of bird. Secondary man to man infections, contact with infected canaries or foreign birds, or indirect contact with clothing worn in an infected aviary have resulted in 9 additional cases. Ten of the 54 infections ended fatally. Before and after the recognition of these facts psittacosis cases attributed to shell parrakeets from California have been observed in Kansas City, Portland, Oregon, New York City, Chicago, Boise, Idaho, Madison, Wisconsin, Massachusetts, Michigan, Minnesota and New Jersey. The details of the epidemiological observations leave no doubt that the budgerigar, either visibly ill or apparently well, may transfer a disease to man indistinguishable from the malady which was attributed to the importation of infected parrots in 1929-1930. Parrot fever, due to indigenous shell parrakeets, has also been noted in England and Germany. It is surprising that the heterogeneous infection chain budgerigar or shell parrakeet—man has not received careful attention since Gordon[1] demonstrated the psittacosis virus in the spleen of a shell parrakeet obtained from the London Zoological Gardens.

Nothing definite concerning the origin or extent of the virus disease in the birds of Southern California is known. Obviously, before planning control measures to protect the public or to save the bird breeding industry from annihilation incident to the various quarantine measures by federal and state organizations it appeared desirable to survey a number of aviaries for the presence of infected birds.

At first, establishments were chosen in which human infections had occurred or had furnished disease-producing birds to the trade. More recently, the population of various aviaries has been sampled by a selection at random of 10% of the birds.

A total of 1,953 shell parrakeets from 66 aviaries has been subjected to detailed post mortem examinations and inoculation tests. Suspensions of the spleens and livers, later only the spleen, the mucous membrane of the nostrils and the filtered content of the cloaca have been injected into mice. When practicable, the spleens of each bird have been examined separately. Experience indicated that, as a rule, spleens measuring less than 4mm. in diameter were non-infectious. Consequently, the spleens of a certain diameter have been pooled and the suspensions in broth have been tested on mice. The presence of the psittacosis virus has been proved by the demonstration of sterile cultures from the enlarged spleen of the birds and mice, the typical anatomical lesions on the liver, the presence of elementary bodies (Levinthal-Cole-Lillie bodies) and by passage experiments of filtrates to mice and occasionally to rice-birds. Approximately 1,850 birds selected according to age in lots of from 6 to 450 originated from different pens of 65 aviaries and pet shops. Over 100 shell parrakeets either dead or sacrificed were collected from 1 aviary in which the epidemic course of avian psittacosis is followed by a daily inspection of the pens. The 1,850 birds were classed by their owners as "healthy" budgerigars. They were chloroformed and the feathers dampened with cresol solution. An external examination showed in a small number of birds a few drops of mucous on the ceres and a soiled anus, more frequently a definite emaciation manifested in a marked atrophy of the pectoral muscles. In the latter class of birds the internal examination revealed a definitely enlarged, heavy and rather tough, slightly saffron or ochre yellow colored liver occasionally with healed necroses and a spleen varying in diameter from 5 to 18 mm. and rarely a few pneumonic patches in one or both lobes of the lungs.

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[1] Gordon, *Rep. on Pub. Health and Med. Subjects,* London, 1930, **61**, 106.

With a few exceptions, the psittacosis virus was readily demonstrated in the organs of birds showing these lesions. However, more common were well nourished shell parrakeets in which merely the size of the spleen attracted attention. In fact, the degree of latent psittacosis infection in a given lot of budgerigars was readily predictable by the percentage of spleens exceeding in diameter the normal average of from 1 to 4 mm. Thus in aviary T a lot of 24 shell parrakeets, in which 14 were found to harbor the virus, and in another, C, a lot of 25 birds with no infection, the sizes of spleens were as follows:

		Aviary T Infected	*Aviary C Non-infected*
Size of the spleen	2 to 4 mm. =	7	24
	5 to 11 mm. =	17	0

Some relationship exists between the age of the bird, the size of the spleen and the latent infection. In a sample of 48 birds from an infected aviary 66% of the old birds had spleens varying in diameter from 1 to 4 mm. and only 13% were larger than 6 mm.; on the other hand, only 50% of the young birds (not older than 5 months) had small spleens while 37% had spleens greater than 6 mm. in diameter. In general, "non-capped" (young) birds are more frequently carriers of the virus than the "capped" or old birds. However, birds 2 to 3 years old may present very large (23x24x9 mm.) spleens resembling a myeloma in which the virus may be demonstrated.

Forty-four or 66% of the 66 aviaries housed budgerigars in which the psittacosis virus was demonstrated in the internal organs. The number of birds found infected in a sample or a pen varied greatly. Occasionally, one sample consisting of old birds revealed no latent infections, while the spleens of a subsequent sample consisting of young parrakeets yielded a high percentage of positive mice. For example, in March only 1 spleen of 6 birds of the

A. R. C. Aviary contained the virus while late in November 186 budgerigars of a sample of 450 presented enlarged spleens and a high incidence of latent infection. A 2% sample of the D. K. Y. Aviary yielded healthy birds in the spring but a 10% sample (31 birds) revealed 4 spleens considered suspicious for psittacosis. The virus was found in 2. The first test on Aviary M. Y. gave one positive bird while the examination of the total budgerigar population of 413 suggested latent psittacosis (22%) in 5 breeding pens. The virus was obtained in the pools of the spleen removed from the birds of every pen except pen 5.

The percentage of virus carrying birds in a sample or in a pen or in an entire aviary varied from 10 to 90%. Incomplete evidence indicates that the virus may be present in the nose or the liver and absent in the slightly enlarged spleen. It is therefore not unlikely that much higher figures would have been obtained if the liver, nose and spleen of every bird had been pooled and tested on mice. The evidence presented probably represents minimum and not maximum percentages. Notwithstanding this reservation it is definitely proved that avian psittacosis infection is a disease of eminently chronic character and as such exists in a high percentage of aviaries. Even breeding places managed under excellent sanitary conditions harbor infected birds. Their existence can only be recognized by a post mortem examination and by mouse inoculations. Judged by the short incubation time (5-6 days), the high fatality rate and the extent of the anatomical lesions produced in mice, a highly active virus in large quantity is usually present.

A study of the epidemiology of the psittacosis disease among parrakeets may answer the question: When are these carriers infectious for man and what factors contribute to human transmissions? It is difficult to correlate the wide and extensive occurrence of psittacosis-virus carrying birds with the relatively low incidence of recognized human infection in population groups which have close contact with birds.

Incomplete observations suggest that the virus leaves the birds much more frequently by way of the nostrils than the cloaca. Furthermore, it may be found in the egg yolk of breeding birds thus suggesting a germinative transmission. The period of autosterilization is not known; it is doubtless of many months duration. Despite the existence of a heavy latent infection rate the spread of the virus is confined to the birds of one pen. Parrakeets housed in an adjacent cage merely separated from the diseased by a wire netting may be anatomically and by inoculation tests entirely healthy. Whether the latency is the sequel, precursor or an intermediary stage of the disease is now the subject of further analysis since exposure experiments with parrakeets from infected aviaries have so far yielded negative results.

6540

Toxicity, Distribution, and Excretion of Thallium.

PAUL A. SHAW. (Introduced by C. D. Leake.)

From the George Williams Hooper Foundation for Medical Research, University of California.

Thallium sulphate administered by mouth to quail, geese and ducks has been found to be fatally toxic in doses of 12, 15, and 30 mg. per Kg. respectively, calculated as thallium metal. Analyses were made of the distribution of thallium in various body tissues by a gravimetric iodide method, and by a new colorimetric method involving liberation of iodine from potassium iodide by thallium, and the estimation of the color intensity of the iodine in carbon bisulphide. The latter method is accurate to within 5% at a thallium concentration of 15 mg. per Kg. Thallium in liver, kidney, heart, and osseous tissue was estimated to be present in a concentration approximately equal to that which had been administered. Muscular tissue, however, was found to acquire a considerable higher concentration of thallium. Fat was found to contain practically none. Analysis of the tissues of a goose dying 15 days after the oral administration of 20 mg. per Kg. indicated a retention of 35-70% of the thallium given.

The first studies on the excretion of thallium were reported in 1890 by James Blake,[1] a physician residing in Middletown, California, who found by spectroscopic examination that thallium was eliminated in all secretions. Quantitative estimations of the rate of elimination of thallium in the urine after oral administration to dogs, by the colorimetric method noted above, indicated that 60% of the total amount given was excreted in 36 days, with a progressive diminishing rate of excretion.

Although thallium is very slowly excreted, and although the edible tissue of birds seems to retain a relatively large amount of ingested thallium there would seem to be no great danger that secondary poisoning in man might result from the eating of game birds poisoned with thallium, since a relatively large amount of such flesh would be necessary to contain a toxic dose of thallium for man.

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[1] Blake, J., *Compt. Rend. Soc. Biol.*, 1890, **1**, 55.

6541

Experimental Infection with Trypanosoma cruzi from Intestine of Cone-nose Bug, Triatoma protracta.

CHARLES A. KOFOID AND FAE DONAT.

From the Zoological Laboratory, University of California.

Kofoid and McCulloch[1] described *Trypanosoma triatomae* from the digestive tract of *Triatoma protracta* from San Diego, California. Its morphological resemblance to *Trypanosoma cruzi* from *Triatoma megista,* the insect vector of Brazilian human trypanosomiasis, was noted, but failure to find the trypanosome in the blood of the wood rat (*Neotoma fuscipes macrotis*) in whose nests infected *Triatoma* were taken, and the failure to infect young white rats by permitting infected bugs to feed upon them, led to the tentative opinion that the trypanosomes of the 2 species of bugs might not be identical. Findings here reported establish the identity of *T. triatomae* of California with *T. cruzi* of Brazil.

Examination of the blood of wood rats (*Neotoma fuscipes annectens*) from the vicinity of Berkeley, California, has shown them to harbor a trypanosome. This parasite, however, has the morphology of *T. lewisi,* the non-pathogenic trypanosome of the Norway rat. *Ceratophyllus fasciatus,* the common rat flea, which infests both the nests and bodies of the wood rats, has been found to contain trypanosomes in developmental stages, identical with those described by Minchin and Thomson[2] for *T. lewisi. Triatoma protracta* also lives in the nests of wood rats from this vicinity, but examinations of its digestive tract have never revealed trypanosomes in any stage of development. Experimental feeding of these bugs on wood rats naturally infected with *T. lewisi,* from the region of Berkeley has in 22 cases given only negative results.

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[1] Kofoid, C. A., and McCulloch, I., *Univ. Calif. Publ. Zool.,* 1916, **16**, 113.

[2] Minchin, E. A., and Thomson, J. D., *Quar. J. Micr. Sci.,* 1915, **60**, 463.

The dilemma thus presented has been at least partially solved by the experimental infection of young albino rats of the Norway-albino hybrid strain in our stock by the intraperitoneal injection of the intestinal contents of a *Triatoma protracta* infected with *T. cruzi* (= *T. triatomae*) from San Diego. *Triatoma protracta* from Berkeley has been infected by feeding it upon animals experimentally infected by inoculation of intestinal fluid of infected bugs from San Diego.

Trypanosomes first appear in the peripheral blood in fresh smears and on stained slides in experimentally infected albino rats on the 16th to 27th day, but only in very small numbers. In the wood rat (*N. f. annectens*) the parasite was first detected on the 45th day after inoculation. In the opossum (*Didelphis virginiana*) the trypanosomes were detected on the 25th day.

Three albino rats which were inoculated intraperitoneally with heart blood from an infected rat, but in whose blood no trypanosomes had as yet appeared, were splenectomized on the 20th, 23rd, and 34th day respectively after inoculation. The trypanosomes appeared in the peripheral blood in the first rat only, on the second day after splenectomy. In the others the peripheral blood remained negative. Another albino rat was inoculated with citrated tail blood from an infected opossum, but its peripheral blood remained negative. On the 13th day after inoculation splenectomy was performed and this was followed by the appearance of trypanosomes in the peripheral blood of the rat 3 days later. An albino rat was inoculated by eating 6 infected *Triatoma protracta,* but its blood remained negative. On the 25th day after inoculation it was splenectomized and 2 days later trypanosomes appeared in the blood stream.

Two mice inoculated with citrated tail blood of a splenectomized albino rat infected by eating parasitized bugs did not show trypanosomes in their peripheral blood either before or after being splenectomized.

Triatoma known to be infected with trypanosomes were allowed to feed upon 15 albino rats, one wood rat, one deer mouse *(Peromyscus),* and 3 guinea pigs, both with and without careful prevention of contamination of the lesion by the feces of the bug, which are often discharged near the site of feeding. In none of the animals thus exposed were trypanosomes subsequently discovered in the peripheral blood.

The trypanosomes in the mammals experimentally infected conform precisely to the accounts given by Chagas[3] and others in morphology, attachment to corpuscles, and tissue invasion. The Leishmania-like stages of the trypanosome occur in heart muscle and are accompanied by the typical degenerative processes.

We therefore conclude that the trypanosome (*Trypanosoma triatomae,* Kofoid and McCulloch) of the cone-nose bug (*Triatoma protracta*) in California is identical with that found in *Triatoma megista* and in other Reduviidae of South and Central America known to be vectors of Brazilian human trypanosomiasis.

The determination of this identity extends the geographical range of the infected insect vector into the Southwestern United States. Mammals naturally infected have not as yet been discovered in this region, but further investigation by splenectomizing suspected mammals in regions where infected bugs are found may be expected to reveal the infection.

6542

Comparative Physiological Action of Quaternary Ammonium Salts of the Epinephrine Series.*

GORDON A. ALLES. (Introduced by C. D. Leake.)

From the Laboratories of George Piness, M. D., Los Angeles, and the Pharmacological Laboratory, University of California Medical School.

The classical studies of Barger and Dale[1] on the relationships between chemical constitution and physiological action of compounds related in structure to epinephrine included 3 quaternary ammonium salts, hordenine methiodide, 3:4-dihydroxyphenylethyl trimethyl ammonium chloride and trimethylaminoacetocatechol chloride. These compounds were found to resemble nicotine, rather than epinephrine, in their physiological actions and the conclusion was tentatively drawn that the relative intensity of these effects paralleled the relative intensity of the sympathomimetic action of the corresponding amino or methylamino derivatives. It became of interest to see if this generalization would hold for other quaternary ammonium salts of the epinephrine group of com-

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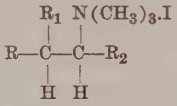
[3] Chagas, C., *Mem. Inst. Oswaldo Cruz,* 1909, **1**, 158.

* Supported in part by the Christine Breon Medical Research Fund.

[1] Barger, G., and Dale, H. H., *J. Physiol.,* 1910, **41**, 19.

pounds, since it might be considered to indicate a close similarity in the processes by which nicotine and epinephrine act upon their respective cellular mechanisms.

Sixteen compounds of this type have been synthesized and studied. Their structures may be represented by the general formula, where R is C_6H_5-,



$$\begin{array}{ccccc} & R_1 & & N(CH_3)_3.I & \\ & | & & | & \\ R- & C & - & C & -R_2 \\ & | & & | & \\ & H & & H & \end{array}$$

p-HO-C_6H_4-, p-CH_3O-C_6H_4-, p-CH_3COO-C_6H_4-, or m, p-$(HO)_2$-C_6H_3-; R_1 is H, OH, $OCOCH_3$ or $OCOC_6H_5$ and R_2 is H or CH_3.

In general, all of these compounds exert a certain degree of activity resembling that of nicotine, with regard to their central nervous system and circulation effects as indicated by the respiratory and pressor responses in dogs following their intravenous injection. Many of these compounds are intensely active, some more active than nicotine on injecting molecularly equivalent concentrations, and the circulatory effects of almost all these compounds in comparison with their central nervous system effects seem to be relatively greater than observed with nicotine. Secondary depression following the stimulant action of this class of compounds appears to be less marked than observed with nicotine, which was also commented upon by Barger and Dale.

Certain generalizations seem to be quite constant in the series of compounds studied and often indicate a lack of parallelism of the effects of substitution with the quaternary ammonium compounds and the corresponding substitution effects with the amino or methylamino compounds. With the present series of compounds, it was observed that: (1) The introduction of a phenolic hydroxyl group in the para position to the side chain has little effect upon the intensity of the action of the quaternary ammonium salts, while two phenolic hydroxyl groups, one in the meta and the other in the para position more than double the activity of the parent phenyl compound. (2) Acetylation of a phenolic hydroxyl group does not markedly change the action of these quaternary ammonium compounds. (3) Methylation of a phenolic hydroxyl group decreases the activity a hundred times or more. (4) The introduction of a α-methyl group, resulting in a 3 carbon atom side chain, increases the activity by 2 times or more. (5) The introduction of an alcoholic hydroxyl group into the β-position of the side chain decreases the activity from 50 to 100 times, or more. (6) Acetylation or benzoylation of a β-hydroxyl group does not markedly alter the activity. (7) The activity of stereoisomers involving asymmetry of the β-carbon atom of the side chain, caused by hydroxyl substitution, are not very different.

No analysis has been made of the exact mechanisms of action of these various compounds other than their general correspondence of effect with that of nicotine. Probably certain differences in the mechanisms of their effects will be found after further study but the quantitative picture of total respiratory and circulatory activities seems to be clearly related to the structure of the compounds studied and further work is in progress to define more completely these relationships.

6543

Methylene Blue as an Antidote to CO Poisoning.*

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This paper is a continuation of the study of the use of methylene blue in antagonizing the effects of CN and CO poisoning in whole animals[1,2].

In these experiments CO was administered to rabbits which had been tracheotomized. A cannula was inserted in the trachea which was then directly connected with the vessel containing the measured CO. The inspired air was separated from that expired by means of a valve. The experiments were done in two parts:

1. Injections of 1 cc. of .01% methylene blue in Ringer solution per kilo body weight were made intravenously a few minutes before CO was administered. CO (10% by volume in air) was then given until the animal stopped breathing. It was found that the peak of the variation curve for control animals was at 4.5 minutes where 39% stopped breathing, whereas the peak of the curve for those animals receiving methylene blue treatment, was at 6.5

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* Aided by grants from the Board of Research of the University of California and by Dr. J. C. Geiger, Director of Public Health of City and County of San Francisco.

[1] Brooks, M. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1228.

[2] Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145.

minutes where 36% stopped breathing. In other words, those animals receiving methylene blue treatment breathed 44% longer than the controls. There were 28 controls and 26 treated animals.

2. Another group was allowed to breathe air containing 8% CO until respiration had ceased. The controls were then given artificial respiration by means of an automatic respirator to see if recovery took place. The remainder were given an intravenous injection of methylene blue and artificial respiration. In the controls 20% recovered, while in the treated animals 92% recovered. In this group 25 controls were used and 20 treated animals. The dye was not effective if injected after circulation had stopped. It was made up fresh for each set of experiments and warmed before injecting.

These experiments confirm previous findings[1,2]. They show definitely that methylene blue assists the animal in overcoming the effects of CO poisoning. It is very probable that methylene blue acts as a temporary catalyst[3] in place of the bound hemoglobin[4, 5] until normal respiration is again restored.

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[3] Barron, E. S. G., *J. Biol. Chem.*, 1929, **81**, 445.

[4] Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1930, **227**, 245.

[5] Warburg, O., and Christian, W., *Biochem. Z.*, 1932, **254**, 438.

Missouri Section.

St. Louis University School of Medicine, December 14, 1932.

6544

Effect of Sodium Bromide on Thyroid Gland of the Guinea Pig.*

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It has been shown that iodine has a stimulating effect on the thyroid gland.[1, 2, 3, 4, 5, 6] It intensifies compensatory hypertrophy of the partly extirpated gland and it increases markedly the mitotic proliferation of the normal gland; it also increases somewhat the size of the acinus cells, softens to a moderate degree the colloid, and increases very much the number of phagocytes which invade the colloid. Considering the chemical similarity of bromine and iodine and their relationship in the periodic system of elements, it was of interest to determine whether bromine has a similar stimulating effect upon the thyroid.

Our experiments were carried out in accordance with methods previously used in our laboratory. Guinea pigs were injected intraperitoneally with NaBr solutions of various concentrations on consecutive days during a certain period of time, and the number of mitoses were counted. The size and character of the acinus cells and of the acini, and the consistency of the colloid and the presence or absence of phagocytes were observed.

A preliminary experiment was carried out to determine the range of effective doses. Five 200 gm. guinea pigs were injected daily intraperitoneally with .05, 0.1, 0.2, and 0.3 and 0.5 gm. NaBr in

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* These investigations were in part carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, *J. Med. Res.*, 1920, **41**, 481.

[2] Loeb, Leo, *J. Med. Res.*, 1920, **42**, 77.

[3] Loeb, Leo, *Am. J. Path.*, 1926, **2**, 19.

[4] Loeb, Leo, *Endocrinol.*, 1929, **13**, 1.

[5] Gray, S., and Loeb, Leo, *Am. J. Path.*, 1928, **4**, 3.

[6] Rabinovitch, J., *Am. J. Path.*, 1928, **4**, No. 6; 1930, **6**, No. 1.

aqueous solution over a period of 10 days. The 2 animals receiving the largest doses, namely, 0.3 gm. and 0.5 gm. died on the third day. Thus the succeeding experiments were limited to doses below 0.3 gm. NaBr. The results are shown in Table I.

TABLE I.

Control Animals		Injected Animals		
Weight	Mitoses	Weight	Dose NaBr	Mitoses
165	144	170	10 days 0.05 gm.	1570
165	195	225	10 days 0.1 gm.	5326
		165	10 days 0.2 gm.	0
Av.	169		Av.	2299

In the first series of experiments, guinea pigs weighing 150 gm. and 200 gm. were used and .05 gm. and 0.1 gm. doses of NaBr

TABLE IIa.

Control Animals		Injected Animals		
Weight	Mitoses	Weight	Dose NaBr	Mitoses
140	140	175	8 days 0.05 gm.	254
135	85	155	8 days 0.1 gm.	504
125	82	165	8 days 0.1 gm.	1026
160	296	140	8 days 0.1 gm.	228
145	276	165	15 days 0.1 gm.	262
Av.	176		Av.	475

TABLE IIb.

Control Animals		Injected Animals		
Weight	Mitoses	Weight	Dose NaBr	Mitoses
195	208	195	15 days 0.05 gm.	350
200	162	200	15 days 0.05 gm.	254
195	44	225	8 days 0.10 gm.	120
225	0	220	8 days 0.10 gm.	274
230	390	210	8 days 0.10 gm.	5430
		200	8 days 0.10 gm.	7632
		215	15 days 0.10 gm.	280
		215	15 days 0.10 gm.	2084
Av.	161		Av.	2055

TABLE IIc.

Control Animals.	
Weight	Mitoses
390	36
405 ♀	578
405 ♀	620
410	57
Av.	320

were injected for periods of 8 and 15 days. The results are shown in Table IIa and Table IIb.

At this time a group of guinea pigs weighing 400 gm. was added to the control series to determine whether mitotic activity may vary with the size of the animal. (Table IIc.) In this group the average number of mitoses is higher than those of the groups with lower weights. This may be due to the 2 females, which had 578 and 620 mitoses respectively. The remaining experiments were limited to male animals, since it was possible that cyclic changes in female guinea pigs might have some effect on thyroid activity.

The animals injected with NaBr showed increased activity in the thyroid gland, and wide variations in the number of mitoses are observed. However, the smallest number of mitoses in the injected animals is larger than the average number found in the controls.

In another series, animals weighing 150, 200, and 400 gm. were injected for 10 days with doses of NaBr varying between .025 and .2 gm. As recorded in Table III, the largest number of mitoses were found when .05 gm. doses were given to 150 gm. guinea pigs, .1 gm. doses to 200 gm. guinea pigs and .15 gm. doses to 400 gm. animals.

TABLE III.

Weight 150 gm.—10 days.		Weight 225 gm.—10 days.		Weight 400 gm.—10 days.	
Dose	Mitoses	Dose	Mitoses	Dose	Mitoses
.025	110	.025	84	.025	76
.5	496	.05	325	.05	125
.1	380	.1	912	.1	220
.15	240	.15	400	.15	340
.20	96	.20	150	.20	108
Av.	264	Av.	374	Av.	174

In a further series 200 gm. animals were injected with doses of .05 to .25 gm. NaBr for 6 and 8 days. The results are shown in Table IV.

TABLE IV.

Weight 200 gm.—6 days.		Weight 200 gm.—8 days.	
Dose	Mitoses	Dose	Mitoses
.05	398	.05	608
.1	188	.10	140
.15	346	.15	225
.20	230	.20	28
.25	100	.25	1264
Av.	252	Av.	453

TABLE V.

Control Animals Weight 180-210 gm.—10 days. Mitoses	Injected Animals—.1 gm. NaBr. Weight 180-210 gm.—10 days. Mitoses
105	452
56	288
88	176
116	312
68	406
24	524
84	128
92	
Av. 80	Av. 327

TABLE VI.

Control Animals Mitoses	Injected Animals—.05-.15 gm. NaBr. Mitoses
144	1570
195	5326
140	254
885	504
82	1026
296	228
76	262
208	350
162	254
44	120
0	274
390	5430
36	7632
578	280
620	2084
105	496
56	380
88	240
116	325
68	912
24	400
84	125
92	220
	340
Av. 158	398
23 animals.	188
	346
	608
	140
	225
	452
	288
	176
	312
	406
	524
	128
	Av. 897
	37 animals.

In the last series, guinea pigs weighing 200 gm. were injected .1 gm. NaBr over a period of 10 days. This dose and length of time of injection appear to be the optimal as indicated in the preceding experiments. The results recorded in Table V show that the average number of mitoses for the control group is 80, whereas that for the injected group is 327.

In Table VI, all the controls used in the various experiments are grouped together, and the animals injected with effective doses of NaBr ranging between 0.05 and 0.15 gm. form a second group. The average number of mitoses in the controls is 158, in the injected animals, 897. We determined the probability that these results are not due to a coincidence and found the probability to be about 60:1.†

Effect of NaBr on the size of the acinus cells and the character of the colloid. Histological examination in every case revealed a definite increase in size of the cells in the injected animals. The sizes were determined accurately by measuring with a micrometer scale and were found to be from 0.8 to 1.2 mu for the controls and from 1.3 to 2.2 mu for the injected animals.

The colloid in the injected animals was in general, soft, staining pale red with eosin, and containing numerous peripheral vacuoles. Lymphocytes and phagocytes are not seen. In this respect the glands differed from those of animals which had received injections of iodide, in which the number of phagocytes is considerable.

Swingle[7] studying the effect of various substances on the metamorphosis of anuran larvae observed that whereas diiodotyrosine induced a complete metamorphosis within a short time, dibromtyrosine was ineffective. In a second experiment, however, he found a slight effect with dibromtyrosine and he concluded, therefore, that bromine in this case was not entirely inert although it cannot take the place of iodine. After the completion of our experiments we found that Minowada[8] recently fed potassium bromide to pigeons and noted that the thyroid showed a state of high functional activity. However, his results are based on only a qualitative histological study.

Conclusion. We may, therefore, conclude on the basis of quantitative studies that bromine in the form of NaBr exerts a definite stimulating effect on the mammalian thyroid as manifested in the increase in mitotic figures, a slight but definite softening of colloid, and an increase in the size of the acinus cells. However, this effect is not as marked as that produced by iodine, inasmuch as with the latter the average number of mitoses is about 5 times greater than that in the animals treated with NaBr.

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† We are indebted to Professor Paul Rider for his advice in the determination of these figures.

[7] Swingle, W. W., *Biol. Bull., M. B. L.*, 1923, **45**.

[8] Minowada, M., *Acta Dermat.*, 1928, **2**, 385.

6545

Action of Theelin on the Domestic Fowl.

J. B. MITCHELL, JR. (Introduced by John Auer.)

From the Department of Pharmacology, St. Louis University School of Medicine.

Juhn and Gustavson[1] have demonstrated the effectiveness of extracts of human and pig placentae, and human pregnancy urine in altering to the female type the new-growing feathers of Brown Leghorn cocks and capons. These authors have also pointed out a positive oviduct response to the same extracts in the immature female.

The present paper reports some effects of the injection of the crystalline female hormone, theelin, into the ovariectomized Brown Leghorn fowl. The writer wishes to acknowledge his indebtedness to Dr. E. A. Doisy and the Department of Biochemistry of this university for helpful suggestions and for bio-assay of material, and to the Department of Pathology for the preparation of tissues for microscopic examination. Full credit and thanks are due also to Parke-Davis and Co. for their very generous supply of theelin. The birds used were pure-bred Brown Leghorn stock, ovariectomized at 3 months of age, and examined periodically thereafter for weight, plumage, comb, and other changes. Autopsy was done on all, with special gross and microscopic attention to gonads and accessory sex characters.

Recalling the work of Domm,[2] the female fowl after a successful ovariectomy assumes the male type of feathering, spurs, and head furnishings, the latter being contingent upon the development of a compensatory testis-like gonad on the right side. The following results present some of the effects of theelin when injected under these conditions:

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[1] Juhn and Gustavson. *J. Exp. Zool.*, 1930, **56**, No. 1.

[2] Domm, L. V., *J. Exp. Zool.*, 1927, **48**, No. 1.

Twenty-two birds were subjected to 5 experimental procedures: a qualitative and a quantitative test of the effectiveness of an olive oil solution subcutaneously injected, a test of a saline solution, a more prolonged injection of oil solution, and finally an injection of saline solution in divided doses.

Results. Injection of an olive oil solution of theelin in a dosage as low as 20 rat units daily is sufficient to cause a change of new-growing feathers from male back to female type, as Juhn and Gastavson described for the capon. Alternate bands may be produced in these feathers by alternating injection periods with non-injection periods. A saline solution of theelin in similar dosage is without effect in altering this feather reaction. A dosage of 150 rat units per day, given as 3 injections per day (one every 8 hours) is barely sufficient to change the color of the mid-portion of the feather back to female type. With more prolonged injection (3 weeks) of the oil solution, no change in head furnishings was noted, but autopsy revealed a decided stimulation of oviducts, which average 6 times those of controls in weight, and show gland and muscle development.

Conclusions. 1. The crystalline product, theelin, is quite effective in restoring to female type those secondary sex characters which have been altered by ovariectomy—namely, feathers and oviduct. 2. With 3 weeks' injection, theelin does not interfere with the stimulus to comb-growth afforded by the compensatory right gonad in this species. 3. Olive oil solution is far more effective in producing these results in the fowl than is saline solution, possibly because of the too rapid absorption and elimination of the saline solution.

6546

Comparison of Ciliary Activity under In Vitro and In Vivo Conditions.*

ALFRED M. LUCAS. (Introduced by E. V. Cowdry.)

From the Anatomical Laboratories, Washington University School of Medicine, Saint Louis.

Normal ciliated epithelia removed from the animal body and kept under optimal conditions vibrate vigorously for long periods of time.

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* The expenses for a program of work of which this is a part have been defrayed by grants from the Chemical Foundation, National Research Council, and the Science Research Fund of Washington University.

Epithelium from the frog's pharynx is a classical example of the unceasing vibratility and indefatigability of cilia; it has been kept viable in tissue culture for as long as 56 days.[1] It has therefore been generally assumed from results of methods available for study that cilia under *in vivo* conditions show a similar unceasing activity. McDonald, Leisure, and Lenneman,[2] Seo,[3] and Pohle[4] in their experiments on frog's epithelium *in vivo,* regard the normal state of the cilia as active. The need of methods which will permit direct observation of ciliary activity has been emphasized,[5] and the present report deals with the application of a method by which cilia arranged in a field, as they are in the pharynx of the frog and the nasal cavity and sinuses of higher animals, may be kept under direct observation, thus eliminating the indirect evaluation of movement of inert particles applied to the surface, which method is of value in a study of *direction* of ciliary movement but not in the determination of *extent* or *continuity* of movement. The use of such a direct method reveals that when no stimulating factors are present the cilia are not active.

A strong concentrated beam of light passed through a water filter is thrown on the ciliated surface at as an acute angle as possible. The microscope is focused upon one of the many "high-lights" reflected. A microscope tube fastened to a sliding rod with adjustable joints permits focusing the microscope at any angle and thus quickly and easily aligning the optical axis with the reflected beam from the "high-light".

Magnification of 78 times with a Spencer 5.4× objective and 20× ocular is ample. The long working distance of 29 mm. permitted with this objective is a decided advantage. The low magnification is adequate because the light directed across the surface of the epithelium causes the ciliary waves to cast shadows and thus appear larger than when they are viewed with transmitted illumination.

A frog† is fastened to an adjustable platform and its lower jaw retracted. Most of the animals were pithed, but for comparison a few were not. The cilia of the pharynx showed no activity a few minutes after the jaw was retracted and frequently this state was reached immediately after the mouth was opened. A mucous layer covered the ciliated surface and it also was inert. The light was not responsible for this quiescent state, because, if it has any effect at all it acts as a stimulus. Occasionally when the lamp has been turned on after the frog has rested in the dim diffuse light of the room there occurs after a few seconds scattered, weak vibratory movements of cilia which formerly were quiescent. Cilia *in vitro,* likewise, according to Umeda[6] are stimulated by light of long wave lengths. Wharton[7] finds, however, on excised trachea that light has an inhibitory effect. Evaporation of water with its consequent drying of the surface is not a factor responsible for the quiescent state of the cilia for many reasons brought out in the following experiments.

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[1] Umeda, T., *Acta Dermat.*, 1926, **8**, 95.

[2] McDonald, J. F., Leisure, C. E., and Lenneman, E. E., *Am. Acad. Ophthal. and Otolaryngol.*, 1928, 318.

[3] Seo, A., *Jap. J. Med. Sc.*, III, *Biophysics*, 1931, **2**, 47.

[4] Pohle, K., *Arch. Exp. Path. u. Pharm.*, 1931, **159**, 452.

[5] Lucas, A. M., *J. Morph.*, 1932, **53**, 243.

† Winter frogs only have thus far been employed.

Fine dry carbon particles of lamp black were delivered from an atomizer upon the quiescent cilia of the pharynx. No reaction was apparent for several seconds, after which a rapid acceleration in activity took place followed by a diminution to a more or less constant rate. The activity sometimes endured until the carbon from regions anterior to the point of observation passed across the field, but more often isolated groups of cilia became temporarily quiescent; soon again resuming an active part in the propulsion of mucus. The mucous film being a viscid elastic sheet as pointed out by Hilding[8] continued movement across the groups of quiescent cilia. The mucous cells likewise appeared to react to the presence of carbon, it seemed by direct observation. The cilia, however, had become active upon the application of carbon before changes in the thickness of the mucous layer became apparent. A rough estimate of the thickness of the mucous layer may be determined by the fact that the ciliary waves are distinct when the layer is thin and disappear from view when the layer becomes sufficiently thick; also particles upon the surface of the mucus cast shadows on the epithelium, which criterion also may be used as a measure of thickness.

The vibratory movements of the cilia soon ceased following the passage of the mucus with its overlying particles. The diminution of activity is not as rapid nor as uniformly distributed as the acceleration and it is possible that following the passage of the carbon laden mucus certain readjustments of the new sheet are involved before the cilia come to complete rest.

Air as well as dry carbon was blown on the pharynx when the atomizer was used. A current of air containing no carbon, therefore, was blown vigorously against the surface; it produced a marked decrease in the thickness of the mucous layer. It occurred too rapidly to be a drying effect and was undoubtedly due to a pushing of the semifluid material outward in all directions. The cilia underlying the thinned area of mucus became active several seconds later, showing the same reactions that they did when carbon was included except that this activity lasted for a much shorter period of time—less than one minute to several minutes, dependent upon the strength of stimulus, the length of time the animal has been under observation, et cetera. When the cilia again assumed a quiescent state the mucous layer was apparently as thick as it was before.

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[6] Umeda, T., *Acta Dermat.,* 1927, **10**, 603.

[7] Wharton, D. R. A., *Am. J. Hyg.,* 1931, **14**, 109.

[8] Hilding, A., *Arch. Otolaryng.,* 1932, **15**, 92.

The same frog was subjected to carbon or blasts of air repeatedly over a period up to an hour with the same sequence of events in each trial. Thus the cessation of activity is not the result of treatment nor can the absence of movement apparent after the mouth is opened, be ascribed to drying effects.

Any possible mechanical effect due to currents of air was eliminated by dusting on the carbon through a cloth bag. Relatively large flakes fell upon the surface instead of fine particles obtained with the atomizer. The cilia became active as before after an interval of about 5 to 10 seconds and ceased activity when the carbon had passed. It is emphasized that cilia were seldom continuously active, but irregular areas and groups during the removal of the mucous coat repeatedly alternated from active to quiescent conditions, nor was the evidence clear that there was any marked predominance of activity immediately underlying the separate flakes of carbon. The irregularities in activity of groups of cilia are in agreement with the variable results of Galtsoff[9] in which the total work accomplished by the cilia under *in vivo* conditions is measured.

Evidence that response may be localized was obtained when a crystal of camphor was placed on the surface of the pharynx anterior to the field of view. The cilia became active in front of the crystal for a distance of several millimeters and their movements ceased a short distance behind the passing object. The stoppage of the cilia was not due to the camphor because the events could be repeated when the crystal was lifted from the posterior end of the pharynx and again placed at the anterior end.

The reaction to air and carbon in the unpithed frog were the same as those found in the pithed animal, except that respiratory movements added a complicating stimulus to the cilia. The slight tension exerted on the pharynx mucosa which accompanies the opening and closing of the glottis had no effect on the cilia, but depression of the eyes which bulged the mucosa quickly stimulated the cilia to activity, followed by a gradual slowing and if the interval between movements of the eyes were long enough the cilia ceased their movements completely. It remains to be determined whether this effect is entirely due to the mechanical factors of the stimulus.

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[9] Galtsoff, P. S., *J. Gen. Physiol.*, 1928, **11**, 415.

Prodding the surface of the mucosa with an instrument will elicit a localized response. The spread of activity from the point touched is related to the strength of stimulus and the sensitivity of the epithelium.

Cessation of the heart beat and absence of blood flow through the mucosa is soon followed by failure of the cilia to respond to the stimuli applied. The same results come about more gradually during the progress of experimentation, probably because the lungs cease to function when the mouth is held open. Although the frog respires through its skin, at room temperature (25-26°C.) gaseous exchange by this means is probably insufficient.

The manipulations already described were repeated under *in vitro* conditions. The excised mucosa of the pharynx spread on a thin piece of glass was examined in a moist chamber. Care was taken that no fluid came in contact with the ciliated surface and that none of the ciliated edges were curled under against the glass. The cilia ceased several minutes after the handling. The surface was subjected at different times to carbon from the atomizer, air from the atomizer and carbon dusted from the cloth bag. The responses were practically the same as they were *in vivo*. When the cilia again assumed a quiescent state after stimulation the mucous layer was as thick as it was originally. Minor differences between ciliary behavior and mucous flow *in vivo* and *in vitro* may be attributed perhaps to the absence of blood circulation in the latter condition.

If the mucous membrane is removed from the pharynx after the experimental manipulations no longer produce the characteristic reactions, and placed on a slide with Ringer's solution and a cover slip, the cilia become vigorously active. Blood corpuscles and debris are rapidly swept along the surface. The cilia beat metachronously and the whole picture is the same as that described for studies on ciliated epithelium in an extraneous fluid medium. Again it is evident that cessation of activity is not due to any impotency in the ciliated cells themselves. The details of the effects produced by

Ringer's *in vivo* are difficult to determine because the "high-light" is reflected from the surface of the added fluid and not from the cilia. When particles are present, however, they can be followed. Their movement becomes more rapid after the aqueous solution is added, which agrees with Hill's observation[10] that the removal of mucus and addition of Ringer's solution greatly increases the rate of movement of particles in the excised trachea.

The results have demonstrated that the cilia of the frog's pharynx in the absence of extraneous factors maintain an inactive quiescent state, that with mechanical stimulation due to movements of the eyes or prodding with an instrument or addition of inert particles or derangement of the mucous layer, the cilia are stimulated to activity which ceases when the cause is removed. Therefore, the base line for physiological studies of ciliary behavior *in vivo* and even *in vitro* is zero for the frog's pharynx and not some value obtained from the rate of particle movement over the surface, nor after the surface has been washed with Ringer's solution. Thus it is evident that the effects on ciliary activity reported for many drugs, solutions, gases, et cetera, have been learned from an epithelium which was already acting under the influence of one or more stimuli and it may be that some of those agents which are said to have no effect on ciliary movement may act as a stimulant when the normal base line of activity is taken at zero.

6547

Nature and Function of Certain Fibers of the Vagus—A New Concept in Peripheral Nerve Organization.

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An analysis of the vagus nerves of the cat and turtle (Heinbecker[1]) demonstrated 3 distinguishable potential complexes. The first had physiological properties characteristic of somatic nerve fibers, the other 2 had properties of a much slower order, properties which our subsequent investigations have associated with autonomic motor functions. A correlation between potential form and nerve fiber type as revealed in osmicated cross sections of nerve indicates that the first potential complex is derived from larger myelinated fibers, the second from small thinly myelinated fibers and the third from non-myelinated fibers.

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[10] Hill, L., *Lancet*, 1928, **215**, 802.

[1] Heinbecker, P., *Am. J. Physiol.*, 1930, **93**, 284.

Experiments have been performed on the cat in which stimulating and recording electrodes were placed on the central portion of one cervical vagus nerve in an animal in which both nerves were cut. The potential record of the nerve was observed coincidentally with the physiological effects in the animal resulting from electrical stimulation. The afferent fibers whose activity is responsible for respiratory and vascular reflex effects are found to fall in the group giving rise to the first potential complex. No further effects are elicited by stimuli strong enough to excite the fibers giving rise to the second and third potential complexes.

The vagus nerve above the nodose ganglion was sectioned in cats and after sufficient time for degeneration the vagus nerve trunk in the neck was studied functionally. In such preparations adequate stimuli failed to cause slowing of the heart rate, but normal results from the fibers responsible for certain motor effects in the lungs and duodenum were still obtained.

Section of vagus roots at their exit from the brain stem after sufficient time for degeneration had a similar effect, indicating that the fibers responsible for slowing of the heart rate have their cells of origin within the central nervous system.

Experiments in normal animals in this and previous work (Heinbecker[2]) have established the fact that vagus efferent fibers to the heart, lungs and intestine are non-myelinated. These and further studies, histological and functional, have shown that the cells of origin for such fibers motor to the lungs and intestine, unlike those to the heart, have their cells of origin in the nodose ganglion. *They have a central and a peripheral process and no evidence of a synaptic junction in this pathway through the nodose ganglion is demonstrable.* Still further experiments have excluded the possibility of the fibers in question being sympathetic in origin.

These experimental results establish the fact that in the vagus nerve motor fibers exist whose cells of origin lie outside the central nervous system. Heinbecker, O'Leary and Bishop[3] have shown that unmyelinated fibers with physiological properties similar to those of unmyelinated motor vagus fibers have their cells of origin in the spinal ganglia and these are the fibers which upon stimulation cause vasodilatation. It has been customary to characterize the activity of such fibers in the dorsal roots as antidromic. Inasmuch as such an explanation could not possibly be applied to similar fibers of the vagus nerve, the existence of true antidromic effects is questioned. Our findings would seem to impose a limitation on the general applicability of the Bell-Magendie hypothesis with regard to the nature of fibers whose cells of origin are in ganglia, other than sympathetic, outside the central nervous system.

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[2] Heinbecker, P., *Am. J. Physiol.*, 1931, **98**, 220.

[3] Heinbecker, P., O'Leary, James, and Bishop, George H., in press.

6548

Further Evidence of the Virus Nature of Interstitial Bronchopneumonia.

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Interstitial bronchopneumonia in man is a characteristic feature in 3 epidemic diseases, measles, epidemic influenza, and whooping cough. In an experimental study of the pneumonia produced by vaccine virus, Muckenfuss and I[1] have shown that the interstitial tissue of bronchial and alveolar walls is thickened by an infiltration of mononuclear cells. In this respect vaccine virus pneumonia resembles interstitial bronchopneumonia in man, although the experimental lesion is not complete, because it lacks the exudate of polymorphonuclear leucocytes and fibrin commonly found in the lumen of the affected bronchi and surrounding alveoli. We have described[2] a method of reproducing the typical interstitial bronchopneumonia, as seen in man, by injecting vaccine virus into the lungs of animals and introducing, a day or 2 later, into the same animals, a suspension of pyogenic bacteria. The results strongly suggest that interstitial bronchopneumonia is the result of the combined action of a virus and bacteria; the virus producing the interstitial cellular infiltration and the bacteria calling forth the acute inflammatory exudate in the bronchus and neighboring alveoli.

Of the 3 human diseases associated with interstitial bronchopneumonia, measles is the only one now generally accepted as a virus disease. The etiology of epidemic influenza is unknown, but many investigators have suggested that it is caused by a virus; and in an anatomical study of this disease we have cited the similarity of the pulmonary changes to those of virus pneumonia in animals as indirect evidence in support of such a view.[2] Whooping cough, on the other hand, is universally regarded as a bacterial disease, due to the Bordet-Gengou bacillus. If a virus is necessary for the production of interstitial bronchopneumonia, as we maintain, on the basis of our experimental work, it was imperative that evidence be adduced for the presence of such an agent in whooping cough in order to account for the type of pneumonia that so frequently complicates this supposedly bacterial disease. I reported the finding of intranuclear inclusions in 12 out of 35 cases of pertussis and their absence in all but 2 children of a control series of 90 autopsies.[3] The pneumonia associated with pertussis, on account of its interstitial character, was pointed out as being similar to that seen in other virus diseases, as well as to the experimental vaccine virus pneumonia that we had reported.[4] The presence of these inclusions has since been confirmed by Rich,[6] but he reports finding them in only 4 of his cases, and suggests that they are the result of aspirated herpes virus. We have injected an emulsion of tissue from several cases containing these inclusions into the brain, eye, or testicle of rabbits but never observed herpetic lesions in these animals. In experiments upon apes this investigator and his associates[5] have produced a catarrh of the upper respiratory tract by injecting bacteria-free material, filtered sputum and citrated blood, from children ill with pertussis, but they regard the agent as the virus of the common cold or some hitherto unrecognized virus which acts as the primary infecting agent in whooping cough.

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[1] Muckenfuss, R. S., McCordock, H. A., and Harter, J. S., *Am. J. Path.*, 1932, **8**, 63.

[2] McCordock, H. A., and Muckenfuss, R. S., *Am. J. Path.*, March, 1933, **9**.

This communication records the presence of interstitial bronchopneumonia in dogs during the course of spontaneous distemper. I assisted Roman in a study of canine distemper,[7] which definitely established the fact that distemper is due to a virus and is not a bacterial disease produced by *B. bronchisepticus*. This was confirmed by Dunkin and Laidlow.[8] Roman was primarily interested in the lesions of the nervous system, while I studied the bacteriology and the pathological anatomy of the pulmonary manifestations in spontaneously infected animals.

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[3] McCordock, H. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1288.

[4] Muckenfuss, R. S., and McCordock, H. A., *Am. J. Path.*, 1931, **7**, 552.

[5] Rich, A. R., *et al.*, *Science*, Oct. 7, 1932, **76**, 330.

[6] Rich, A. R., *Bull. Johns Hopkins Hosp.*, Dec., 1932, **51**, 346.

[7] Roman, B., and Lapp, C. M., *Bull. Buffalo Gen. Hosp.*, 1925, **3**, 40.

[8] Dunkin, G. W., and Laidlow, P. P., *J. Comp. Path. and T.*, 1926, **39**, 213.

The records of 21 dogs and 5 silver foxes studied at that time are still available; of these 15 showed interstitial bronchopneumonia. In an additional recent series, 6 out of 8 dogs with distemper presented pulmonary lesions similar to those found in man following influenza, measles, or whooping cough. The lungs of dogs with typical interstitial bronchopneumonia show, on section, small grayish nodules encircling the smaller bronchi and often surrounded by red hemorrhagic areas. Patches of lobular consolidation are also frequently found. These vary greatly in size and occasionally become confluent and may cause an entire lobe to appear almost uniformly solid. Pleurisy, empyema, and small abscesses have also been seen. In the more acute examples of the disease dark red to purplish areas of lobular consolidation, similar to those seen in early influenzal pneumonia in man, are found, most frequently in the lower lobes.

Microscopically, the changes are practically the same as those encountered in man in interstitial bronchopneumonia. The mildest change in a bronchus consists of an edema and an infiltration of the wall with large mononuclear cells, lymphocytes and plasma cells. A plug of exudate consisting of polymorphonuclear leucocytes, fibrin and bacteria is often found in the lumen of an involved bronchiole, and a similar exudate is present in adjacent alveoli. In more advanced lesions, the epithelium of the bronchus is either partly or completely destroyed and the necrotic debris may fuse with the bronchial exudate. In some instances the entire bronchial wall, with the exception of the cartilaginous plate in the case of larger bronchi, becomes necrotic and an abscess-like mass of leucocytes, bacteria and dead tissue marks the site of the destroyed bronchus. The alveoli about these severely involved bronchi frequently contain dense fibrinous plugs. Large mononuclear cells also are found in the alveoli as well as giant cells, as described by Hecht, and by Karsner and Myers in children following measles or whooping cough. Areas of confluent lobular pneumonia, or simple lobular patches of leucocytic consolidation, are frequently found. Large areas of necrosis have also been observed, and such necrotic tissue is usually sprinkled with colonies of bacteria, which often are Gram-positive cocci.

The dark red areas of lobular consolidation seen in acute cases are found on section to consist in large part of necrotic material together with hemorrhage into the alveoli. These areas somewhat resemble recent hemorrhagic infarcts. Similar lesions are found in individuals dying a few days after the onset of symptoms of epidemic influenza, and we have described them in acute vaccine virus pneumonia and have applied the term "hemorrhagic virus pneumonia" to this condition.

Conclusion. The presence of interstitial bronchopneumonia and associated lesions in spontaneous cases of canine distemper, a known virus disease, supports and strengthens our previously published conclusion that this type of pneumonia is in all probability produced by the combined action of a virus and bacteria.

6549

The Examination of Neoplasms of the Breast and Skin by the Method of Micro-incineration.

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An investigation of the inorganic salt content of neoplasms seemed appropriate since the technique of micro-incineration, recently adapted to cytological problems (Scott[1]) offers a precise method for ascertaining whether the mineral salts in malignant tissues differ in kind and amount from the normal. An examination by micro-incineration of those questionable lesions, considered by many as precancerous or having some relation to subsequent malignant change, seemed of especial interest. This report is limited to an investigation of neoplasms of the breast and skin, including mucous membranes covered with squamous epithelium, by the method described by Policard[2] and by Scott.[3]

Breast. The ash obtained by micro-incineration of a section of normal resting breast is flat white, indicating (according to Policard) the presence of calcium. The residue is more dense in the epithelium lining the ducts and alveoli than in the connective tissue. In the former the original cell outlines are readily determined because of the heavy ash concentration in the nuclei. Here the particles may vary in size, while in the cytoplasm minerals are deposited as fine, uniform stipplings. In the stroma calcium is deposited in long, punctate strands, conforming to the arrangement of collagenic fibers.

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[1] Scott, G. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 349.

[2] Policard, A., and Okkels, H., *Anat. Rec.*, 1930, **44**, 349.

[3] Scott, G. H., *Am. J. Anat.*, in press.

In the varied lesions known as "chronic cystic mastitis", marked differences were observed, corresponding in degree with the state of the duct and alveolar epithelium noted in stained control sections. In that type of chronic mastitis with cystic enlargement of the ducts and no evidence of proliferation of the duct epithelium, the residue of the ducts, as well as that of the stroma, is white. In the stroma it is possibly in greater than normal concentration. Where one sees, in the stained section, in addition to cyst formation, evidence of epithelial proliferation, the character of the ash in the incinerated section changes decidedly. Epithelial cells showing proliferative changes, upon incineration leave a decidedly bluish ash and a decreased amount of white ash. Likewise in every carcinoma of the breast, no matter what type, this blue color of the mineral ash of malignant epithelial cells was pronounced. In such cells the flat white ash seen in the normal breast or in cases of chronic cystic mastitis with no epithelial proliferation was markedly decreased. This color change was also noted by Policard and Doubrow.[4]

The ash of stroma in carcinoma of the breast varies from flat white to bluish white. Where there is epithelial degeneration the blue color decreases or is lost and an intensely white ash remains. The sarcoma of the breast examined showed areas of cellular degeneration in which no blue ash was seen. In the cellular areas the ash was bluish-white, but not as concentrated as in carcinoma.

Skin. As pointed out by Scott (1933, loc. cit.), the epidermis gives a decidedly bluish ash. This is probably the only tissue normally showing this property. The ash of the corium and underlying structures is white. The upper layers of the epidermis show white ash only.

Numerous warts and hyperkeratoses resulting from X-ray burns were examined. The blue color of the hyperplastic epithelial cells was intensified, although they also showed granules of white ash, mainly in the nuclei. The desquamated, intensely cornified epithelium did not show this blue color, but was of the dense, flat white seen in normal desquamating epithelium. The epithelial pearls gave a less abundant white ash. The underlying corium and adipose tissue showed mostly calcium ash.

In the naevus, whether pigmented or not, the covering epidermis has the same appearance as normal skin. In marked contrast is the white ash left by incineration of the nests of so-called naevus cells. Reddish colored ash, indicative of iron, is not seen. When, however, the mole undergoes malignant degeneration and gives rise to a melanocarcinoma, the ash acquires a bluish color in addition to the large amount of flat white ash.

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[4] Policard, A., and Doubrow, S., *Ann. d'anat. Path.*, 1924, T. 1, 63.

Carcinomata of the skin whether of basal or squamous cell type, show a greater concentration of blue ash in the nests of malignant epithelial cells. This coloration is the same in nuclei and cytoplasm. In squamous cell carcinomata the epithelial pearls and areas of cellular degeneration show a predominantly white ash. The amount of ash decreases in epithelial pearls and increases in areas of cellular degeneration.

Discussion. In incinerated tissues the white ash is either calcium or magnesium. Policard believes it possible that bluish ash indicates the presence of sodium or potassium. Exact determination of this is yet to be made, but with the evidence at hand one may safely say that there is a fundamental chemical difference in the mineral salt content of benign and malignant neoplasms. This differentiation appears in early proliferative changes, considered by some as precursors of malignant neoplasms.

6550

Isolation of a Crystalline Depressor Substance from the Brain.

C. J. WEBER, J. B. NANNINGA AND RALPH H. MAJOR.
(Introduced by E. A. Doisy.)

From the Department of Internal Medicine, University of Kansas School of Medicine.

It has been shown[1, 2, 3] that a depressor substance is present in certain extracts from brain tissue. Major and Weber[2] presented evidence that the active depressor substance was not histamine choline or acetyl choline. Major, Nanninga and Weber[3] presented further evidence that the depressor activity of these brain extracts

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[1] Major, Ralph H., and Weber, C. J., *J. Pharm. and Exp. Therap.*, 1929, **38**, 367.

[2] Major Ralph H., and Weber, C. J., *J. Pharm. and Exp. Therap.*, 1930, **40**, 247.

[3] Major, Ralph H., Nanninga, J. B., and Weber, C. J., *J. Physiol.*, 1932, **76**, 487.

was not due to either histamine, choline, acetyl choline, adenosine or adenylic acid. We have recently obtained from these brain extracts a crystalline compound which possesses a marked depressor action upon the blood pressure of dogs under ether anesthesia. We have employed the following procedure to obtain the active depressor substance in crystalline form.

After removal of the acetone from the crude acetone extract of brain, 60 gm. of potassium carbonate was added to each 100 cc. of the crude extract. This was extracted with an equal volume of 95% alcohol and the extraction repeated 4 times. This, after standing overnight in the ice-box, was filtered; the filtrate evaporated under partial vacuum to a consistency of a thick syrup and then redissolved in 95% alcohol. Concentrated sulphuric acid mixed with 50 parts of 95% alcohol was added to this solution, producing a heavy flocculent precipitate. The sulphuric acid alcohol mixture was added until no further precipitation was obtained, the solution filtered, neutralized with NaOH and again filtered. The filtrate was then evaporated under a partial vacuum to a thick syrupy consistence, dissolved in water and treated with phosphotungstic acid, which produced a copious white precipitate. This precipitate was separated by filtration, the filtrate evaporated under low pressure to the consistency of a thick syrup and then dissolved in absolute alcohol.

This alcoholic solution, after the separation by filtration of a slight insoluble precipitate, was subjected to fractional precipitation with water-free ether. The addition of one volume of ether produced a precipitate which was dissolved in absolute alcohol and then treated with 0.1 volume of ether. This procedure again produced a precipitate which was separated by filtration. The filtrate was treated with 0.3 volume of ether, the precipitate removed and the filtrate then treated with one volume of ether. This again produced a precipitation, the precipitate was dissolved in absolute alcohol and 0.3 volume of ether added. The precipitate forming was dissolved in absolute alcohol and this solution was treated with concentrated sulphuric acid in 50 parts of absolute alcohol until no further precipitation occurred. This precipitate was removed, one volume of ether added to the filtrate, the precipitate again removed and 3 volumes of ether added to the filtrate. This procedure again produced a precipitate which was filtered off and for the sake of convenience we shall designate them as the final precipitate and the final filtrate.

The final precipitate was dissolved in absolute alcohol and treated with 5 volumes of ether. A precipitation occurred and the precipitate was found under the microscope to consist of small needle-like crystals, which were quite soluble in absolute alcohol and could be readily precipitated by 5 volumes of ether.

The final filtrate containing 3 volumes of ether was treated with an additional 3 volumes of ether, which produced a marked cloudiness. On standing in the ice-box long needle-like crystals appeared. These crystals also were readily soluble in absolute alcohol and the addition of 3 volumes of ether again produced the long needle-like crystals.

The small crystals showed a marked depressor activity after 7 recrystallizations from absolute alcohol and no loss of activity could be detected as the result of these recrystallizations. The long needles likewise showed no loss of depressor activity after 6 recrystallizations.

The larger needles after being dissolved in water, the water removed and then being dissolved in absolute alcohol, came down, upon the addition of ether, in the form of small needle-like crystals apparently identical with those obtained from the final precipitate.

Both large and small crystals have the same depressor activity. 0.1 mg. of each kind of crystal caused a fall of 8 mm. Hg. in blood pressure in a dog weighing 12 kilos, under ether anesthesia, while 0.5 mg. produced a fall of 40 mm. Hg. Both types of crystals obtained by precipitation with ether on exposure to the air are rapidly converted into a gummy mass. Aqueous solutions of the crystals give a negative Pauly reaction, a negative Sakaguchi reaction and are quite active in an atropinized dog.

New York Meeting.

New York Academy of Medicine, January 18, 1933.

6551

A Method of Enhancing the Vitamin A Value of Cod Liver Oil.

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Some unpublished data kindly supplied to the authors by Dr. Walter H. Eddy have indicated that cod liver oil in an emulsified form is a more efficient source of the fat soluble vitamins than plain cod liver oil.

Recent comparisons in this laboratory of the vitamin values of emulsified vs. plain cod liver oil yield results in accord with those obtained by Eddy, and show quite conclusively that the emulsified cod liver oil which we have examined is a decidedly more efficient source of vitamin A than the same oil in the unemulsified state.

The particular type of emulsion which has interested us is the same as that employed by Eddy in his earlier investigations, and consists of 22.37% by weight of high grade cod liver oil with 77.63% of concentrated malt extract.* Emulsification of these constituents is effected by thoroughly agitating the cod liver oil and the malt extract in an especially constructed apparatus operated under reduced pressure. A fine emulsion results, with the oil uniformly distributed in the aqueous phase as minute droplets microscopic in size.

While other tests have shown that the malt extract itself contains relatively little vitamin A, it was thought that some factor or factors present in the extract, such as vitamin B or G, might exert a synergistic action of some kind upon the vitamins of cod liver oil and thus increase their effectiveness. We have, therefore, included not only a study of the vitamin A value of the plain as well as the emulsified cod liver oil, but also that of a mixture of the malt extract and cod liver oil.

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* The commercial product Maltine with Cod Liver Oil.

Sherman's[1] method for determining vitamin A was employed. When young rats fed the vitamin A-free diet had ceased to grow and showed by other symptoms that their surplus bodily stores of this vitamin were depleted, they were divided into 3 groups. To one group was fed as daily supplements 4.0 mg. of the cod liver oil emulsion (22.37% cod liver oil by weight); to the second group 0.8948 mg. (22.37% of 4.0 mg.) of the same oil used in the emulsion; and to the third group 0.8948 mg. of the cod liver oil + 3.1052 gm. of the concentrated malt extract. In the case

TABLE I.

Daily Supplement	Rat No.	Survival	Total gain in body wt. end of 35-day test period.
			gm.
4.0 mg. Cod Liver Oil Extract Emulsion (Contains 22.37% C. L. O. by weight)	8258	entire period	21
	8298	” ”	40
	8312	” ”	47
	8348	” ”	43
	8352	” ”	36
	8353	” ”	58
	8517	” ”	50
	8521	” ”	18
	8522	” ”	44
		Average	39.7
0.8948 mg. Plain Cod Liver Oil	8257	entire period	—29
	8259	” ”	— 8
	8261	” ”	20
	8295	23 days	dead
	8296	entire period	—15
	8299	” ”	7
	8313	” ”	—21
	8314	31 days	dead
	8349	25 days	”
	8355	28 days	”
	8515	entire period	13
	8516	” ”	— 9
0.8948 mg. Plain Cod Liver Oil + 3.1052 mg. Malt Extract fed separately	8256	34 days	dead
	8260	entire period	— 1
	8262	” ”	—21
	8292	” ”	—19
	8293	30 days	dead
	8297	entire period	3
	8315	” ”	—41
	8316	34 days	dead
	8350	entire period	12
	8354	” ”	10
	8518	30 days	dead
	8519	30 days	”
	8520	entire period	10

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[1] Sherman, H. C., Chemistry of Food and Nutrition, 4th Edition, 1932, Macmillan.

of the cod liver oil + malt extract supplement, each of these materials was measured as a separate portion but both were consumed almost immediately after they were offered to the test animal.

To facilitate measurement of the small dosages required for the daily feedings, the emulsion was diluted with water, the cod liver oil with peanut oil and the malt extract with water, the aqueous solutions being made to a definite volume before each feeding and dilution of the cod liver oil made at least once each week. All dosages necessary to furnish the required amount of each test material were then carefully measured with a pipette.

The results in Table I and Fig. 1 show the superiority of the emulsion over the plain cod liver or plain cod liver oil + extract fed separately. The animals fed 0.8948 mg. of cod liver oil daily in the form of the cod liver oil-malt extract emulsion made an average gain in weight of 39.7 gm. during the 5 weeks' experimental period. Further, when xerophthalmia existed in the animals of this group at the beginning of the test period, the eye condition became decidedly improved or was entirely cured.

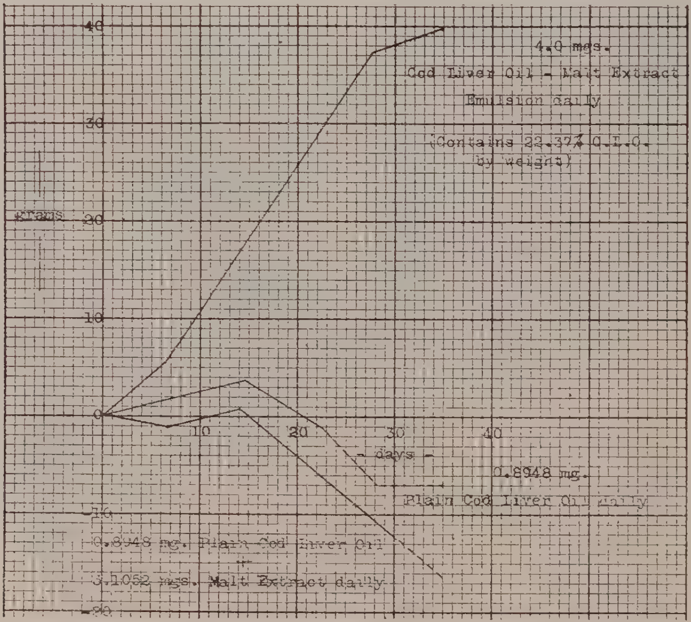

FIG. 1.
Average growth curves of rats fed supplements indicated as their sole source of vitamin A.

In striking contrast to the emulsion-fed animals were those receiving the same amount of cod liver oil supplied as plain oil alone or as plain oil + the concentrated malt extract fed separately. Several of the animals in each of these groups died before the end of the 35-day test period, while those that did live made poor growth or lost weight and were in poor physical condition, with more or less severe xerophthalmia at the end of the period.

From the results there can be little doubt but that the vitamin A present in the oil-malt extract emulsion is much better utilized than that of the plain cod liver oil.

Although the plain cod liver oil + the malt extract fed separately showed no better results than the plain oil alone, thus tending to discount the idea of the synergistic action postulated above, there is still the possibility that such an action may function only when the extract and oil are intimately mixed, *i. e.,* in the emulsified form. Unless it can be shown, however, that the malt extract *per se* plays some part in increasing the value of the emulsified oil, it would appear that the logical explanation for the better results with the emulsified product is that the very fine subdivision of the oil favors a more complete absorption and utilization within the animal body.

6552

Blood Cholesterol in Dogs on an A Deficient Diet.

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Drummond[1] showed that vitamin A was linked to cholesterol and could be separated from it without losing its vitaminic activity. No mention was made that vitamin A might affect the metabolism of cholesterol. Liang and Wacker[2] found that the total cholesterol in rats fed a diet low in vitamin A, fat and cholesterol was greater than in a group of animals fed a diet containing butter and fat. They felt that these results showed that the metabolism of cholesterol was affected by vitamin A. No blood cholesterols are reported. Wieland and his associates[3] suggested that in the normal animal cholesterol is broken up by vitamin A to bile acids and secreted in the bile.

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* The expenses of this investigation were defrayed by a grant from the Josiah Macy Jr. Foundation.

[1] Drummond, J. C., *J. Soc. Chem. Ind.,* 1924, **43**, 928. Drummond, J. C., and Coward, K. H., *J. Soc. Chem. Ind.,* 1922, **41**, 561.

[2] Liang, B., and Wacker, L., *Biochem. Z.,* 1925, **164**, 371.

While studying the effect of A deficiency in young and adult dogs we noted a rise in the blood cholesterol during the period when the symptoms of A deficiency were present. The A deficient diet consisted of cracker meal 100 to 150 gm., lard 20 to 30 gm., vitamins B, G, and D in the form of irradiated yeast and 3 gm. of a salt mixture.[4] In addition the 2 young dogs, 68 and 69, received 100 cc. of boiled milk. When the symptoms of the deficiency were well advanced dog 12 was given carotene and dog 72 was given cod liver oil in the form of the concentrate. The carotene, obtained from the Mead Johnson Laboratories, was put up in maize oil and had an antixerophthalmic potency 10 times that of cod liver oil. The cod liver oil tablets, supplied by the White Laboratories, contained 1000 units of vitamin A. Of the 4 dogs observed, one, 68, died on the 21st day of the diet. Dog 69 was killed on the 41st day of the diet, at which time she showed loss of appetite, listlessness and loss of weight. Dog 12 was killed after having received carotene for 66 days. The livers of dogs 12, 68, and 69 were analyzed for their vitamin A and carotene content. Extraction of the liver oil was done by the method reported by Moore.[5] Determination of the vitamin A content was done by the Carr and Price[6] modification of the Rosenheim and Drummond antimony trichloride test. We have reported the vitamin A and carotene content of the livers of well-fed laboratory dogs.[7] Compared to such animals the vitamin A content of dog 12, fed carotene, is about 1/6 that of the normal animals. The vitamin content of the livers of dogs 68 and 69 was very low. The blood cholesterols were done by the Bloor method.[8]

Table I shows the blood cholesterol findings on 2 young dogs, 68 and 69. In both animals there is a sharp rise which is maintained during the entire A deficient period. In dogs 12 and 72 there is also a sharp rise in the blood cholesterol (Tables II and III) but

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[3] Wieland, H.; Wieland, H., and Mothes, W.; Wieland, H., and Schlichting, O., *Z. f. physiol. Chem. Bd.*, 1924, **134**, 140, 149, 276.

[4] Cowgill, G. R., *Am. J. Phys.*, 1921, **56**, 420.

[5] Moore, T., *Biochem. J.*, 1930, **24**, 692.

[6] Carr, F. H., and Price, E. A., *Biochem. J.*, 1926, **20**, 497.

[7] Ralli, E. P., Pariente, A., Flaum, G., and Waterhouse, A., *Am. J. Phys.*, in press.

[8] Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

TABLE I.

No. days on A deficient diet	Blood Chol. mg.	Symptoms of A deficiency
		Dog No. 68. Young Dog.
Aver. Bld. Chol. prior to diet	185	None
13	303	Loss of appetite
14		Dog began coughing
18		Discharge from eyes
19	[illegible]	Cough pronounced, listless, discharge from eyes profuse
		Dog. No. 69. Young Dog.
Aver.Bld. Chol. prior to diet	223	None
3	262	"
19	303	"
26	317	Loss of appetite for past 4 days
27	370	Dog less lively
29	370	Appetite slightly better
33	370	Loss of appetite more marked
36	317	Refused food. Fed by force
40	317	Quiet. Listless.

TABLE II.

Dog No. 12 Full grown female. Average blood cholesterol prior to diet. [illegible] mg.

Day of A def. diet	Blood Chol. mg.	Symptoms of A deficiency present
[illegible]	[illegible]	Listlessness, loss of appetite, loss of hair, discharging sores over body. Loss of weight.
[illegible]	247	Symptoms same. Refused food for 2 days.

Carotene Period

Carotene cc.	No. days administered	Blood Chol. mg.	Effect on Symptoms of Deficiency
1.25	1	254	No change
	12	153	Greatly improved for 6 days
1.	4	175	Gained 1¼ kg. Coat improved
	5	[illegible]	Improvement continued throughout this period. Appetite excellent.
	12	174	
	15	194	
.5	3	289	Sores reappeared under rear paws. Dog otherwise well
	6	260	Sores gradually improved
	15	256	Dog continued well and lively. Gained ¾ kg.
	16	310	
.25	7	285	Dog apparently well. Coat still slightly shaggy
	13	271	

following the refusal of food there was in each dog a moderate fall. In both these animals when carotene and cod liver oil were given there was definite improvement in the symptoms of A deficiency and a marked drop in the blood cholesterol. In dog 12 when the amount of carotene was reduced the blood cholesterol rose again. Apparently, although the dog had enough of the vitamin to overcome the more marked symptoms of the deficiency she did not have enough to keep the blood cholesterol within normal limits. This is possibly another example of what Sherman[9] refers to when he points out that vitamin deficiencies exist in various degrees. Between an abundance of any of the vitamins in the body and enough to keep the symptoms from being obvious there is plenty of opportunity for the less evident effect of avitaminosis.

TABLE III.

Dog No. 72. Adult female. Average blood cholesterol prior to diet, 198 mg.

No. days on A def. diet	Bld. Chol. mg.	Symptoms of A deficiency
2	196	None
5	202	
7	224	
9	226	
11	215	
13	247	Appetite poor. Loss of weight. From this time on the appetite was capricious, the dog eating only part of each day's meal. The animal was still lively
15	228	
18	212	
20	226	
22	206	Fed forcibly
25	222	
27	208	
29	204	
31	215	Appetite slightly better
36	206	Hair definitely falling from extremities
38	221	
41	226	
44	226	Symptoms pronounced. Sores discharging. Skin scaly
48	333	Pronounced loss of hair. Animal listless
52	303	Sores much worse, breaking down. Eyes discharging
58	278	Eye balls sunken in socket. Animal very quiet
No. days on C. L. O.		Cod Liver Oil Concentrate Started. 10 tablets daily
5	219	Symptoms slightly better
8	185	Eyes much better. Gain of weight
13	188	Appetite much improved
15	190	Dog lively, eating everything. Eyes o. k.

The question, of course, arises as to whether the blood cholesterol might not fall, regardless of the administration of vitamin A. In No. 72 there was a drop of 55 mg. during the 10 day period when the symptoms were becoming worse. Cod liver oil caused a further drop of 93 mg. in 8 days during which time the dog's condition improved. In dog 12, during the time when she refused food, the cholesterol fell 56 mg. After 12 days on carotene, during which time the animals ate well and the symptoms improved markedly, the cholesterol fell 101 mg. In this animal reduction of the amount of carotene was accompanied by a rise in blood cholesterol. The hypercholesteremia apparently occurs while the animal is being depleted of its vitamin stores. When this depletion is advanced the blood cholesterol falls. It may well be that when the animal reaches a state of nutritive failure due to the deficiency and enhanced by the refusal to eat, the blood cholesterol falls because the animal is utilizing its body stores as a source of food. In young animals, dying of an intercurrent infection before profound nutritive failure can occur, the fall in blood cholesterol may not manifest itself. It seemed significant to us that the blood cholesterol was reduced rapidly to normal limits by the administration of the vitamin and the general condition of the animal improved at the same time.

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[9] Sherman, H. C., *J. Am. Med. Assn.*, 1931, **97**, 1425.

Summary. Observations are reported on the level of the blood cholesterol in 4 dogs while on an A deficient diet. There is a rise in the blood cholesterol at the time when the animals developed the symptoms of A deficiency. In the young dogs this was maintained at a higher level than in the 2 older dogs. The feeding of vitamin A or its precursor caused a drop in the cholesterol. When the source of the vitamin was diminished the blood cholesterol rose. While the amount of the vitamin was adequate the blood cholesterol remained at the normal level.

Conclusion. These observations tend to substantiate the suggestion of other[2, 3] investigators that the metabolism of cholesterol is affected by the amount of vitamin A in the body.

6553

Growth-Promoting Rachitogenic Diets for Rats.

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In the Medical Research Council's 1924 "Report on the Present State of Knowledge of Food Accessory Factors" emphasis is laid on the fact that rickets develops in well nourished children and that rachitic dogs also grow at a normal rate while the disease develops.

They call attention to the lack of good growth in rats on all commonly used rachitic diets and say: "It is manifest that experimental rickets in rats as at present produced fails to fulfill the condition of being a disease occurring in well growing and apparently healthy animals." Some of the early diets, made up for other purposes, were highly deficient and gave little growth. Diets designed later on for rickets production were much better in this respect, but even Steenbock's diet No. 2965 falls short of normal growth. This lack of growth cannot be looked upon as a "Vitamin D" deficiency since the addition of cod liver oil in liberal amounts will not materially improve the condition.

Several years ago we began a survey of feeding materials suitable for rickets production with a view to finding a more rational procedure for studies in experimental rickets and avoiding the obviously correct criticism of the British investigators. Numerous experiments were done with a view to satisfying the various aminoacid, vitamin and mineral requirements and it became apparent that Vitamin B (complex) was the most important factor. It was shown that by means of rice polishings a diet otherwise poor in growth and only slightly rachitogenic could be made to produce practically normal growth with a greater degree of rickets. Yeast and yeast extracts gave a marked improvement in growth but led to other complications to be discussed later. Observations on diets with yeast extracts, stressing growth, have been reported by Osborne, Mendel, and Park,[1] and previous to that the subject of growth and rickets has been extensively discussed by Shipley, Park, McCollum, and Simmonds.[2]

The diet we used in the above experiments was:

DIET 746

Casein	5	Rice polishings	7
Egg albumen	5	Milk concentrate	1
Patent flour	76	Cotton seed oil	2
NaCl	2	$CaCO_3$	1
Spintrate	1		

During the 3 weeks of the experiment this gave growth quite comparable to that of a good stock diet.

It will be seen from this that the requirements for growth in rat rickets can be satisfied fully as well as in dog rickets, thereby removing the objections cited. The amount of cod liver oil required for prevention on these more rapidly growing rats with comparatively mild rickets, was the same as in the Steenbock diet for the prevention of extremely marked rickets. The cod liver oil requirement is not *per se* a measure of the severity of rickets. We believe that the conditions are more nearly such that, irrespective of the diet, a certain amount of antirachitic substance is required to turn the metabolism from a rachitic to a non-rachitic state. The amount of phosphate, however, which has to be added to various types of rickets-producing diets to prevent the rachitic changes varies anywhere from about 0.5% of primary potassium phosphate to 2%, depending only in part on the level of calcium in the diet.

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[1] Osborne, T. B., Mendel, L. B., and Park, E. A., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 87.

[2] Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, N., *Johns Hopkins Hospital Bulletin,* 1921, **32**, 159.

Certain practical applications have also been made of this new type of growth-promoting rachitogenic diet. The rickets production is much more rapid and it is possible to produce in 7 days, or less time, an amount of rickets perfectly satisfactory for purposes of "Vitamin D" assay. With one of these diets having Ca/P ratio of .713/.154 = 4.6 the amount of rickets produced in one week as judged by the split, silver-stained tibia is not as much as is obtained on the Steenbock No. 2965, but still is a very suitable degree of rickets to work with.

The diet used had the following composition:

DIET 728

Egg albumen	10.0	Rice polishings	7.0
Wheat gluten	10.0	Milk concentrate	1.0
Corn starch	65.5	Cotton seed oil	2.0
NaCl	2.0	$CaCO_3$	1.5
Spintrate	1.0		

This diet is very suitable for 7-day tests, but does not, necessarily, support growth ideally during a longer experimental period.

In these one-week experiments determinations of inorganic blood phosphate show a mean of 3.7 mg. % for rachitic animals, 4.7 mg. % with partial, and 7.3 mg. % with complete prevention by cod liver oil. The bone ash figures obtained on fat-extracted, dried femurs gave averages of 42% for the rachitic and 46% for the non-rachitic.

We have successfully carried out assays in 7 days, by prevention method, in good agreement with the results obtained by 21-day prevention tests and 31-day line tests (the last 10 days being the test period). We are now engaged in studies looking towards further reduction of time necessary for assay.

6554

Rôle of Bacteria in Decomposition of Plant and Animal Residues in the Ocean.

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A study has been made of the decomposition of marine zoöplankton and of certain marine algae by bacteria in sea water and in marine mud. The formation of carbon dioxide and the liberation of nitrogen in the form of ammonia were used as measures of the rate of decomposition. It was found that the chemical nature of the material has a very important influence upon the rapidity and nature of its decomposition.

Zoöplankton, consisting largely of copepods, and containing 7.9% total N on a dry basis, was found to decompose very rapidly, both in the water and in the mud. Nearly one-third of the total C was liberated as CO_2 within 19 days' incubation, while a large part of the N was liberated as ammonia. A marked difference, however, was observed in the case of the medium in which the decomposition took place, namely, although the amount of N liberated was about the same in the water and in the mud, viz., 40.3 and 38.5 mg., a considerably greater amount of CO_2 was evolved in the mud than in the water, viz., 101 vs. 64 mg., above controls. This difference may be due to the fact that while the nitrogenous constituents of the zoöplankton decompose alike in water and in mud, some of the nitrogen-poor or non-nitrogenous complexes are decomposed more rapidly in mud than in water, probably due to the presence in mud of specific bacteria capable of attacking them.

For the decomposition of the algal material, *Ulva lactuca* and *Fucus vesiculosus* were employed. The decomposition of these substances, also, was quite different in water and in mud media; in Ulva, which contained 1.67% N in the total dry material, there was as much decomposition in water as in mud medium, as shown by the CO_2 liberated, viz., 83.4 and 101.8 mg. C. This was accompanied by the accumulation of small amounts of ammonia, viz., 5.2 and 4.1 mg., respectively, above controls. In the case of the Fucus, which contained only 0.86% N, there was very little decomposition in the water, while considerable decomposition took place in the mud, viz., 10.4 and 85.8 mg. C as CO_2, respectively. The reason for this is very easy to find, when one observes that no N was liberated as a result of the decomposition of the Fucus material by the bacteria. Since the N content of Fucus is very low, considerably less than is needed by the bacteria to bring about its decomposition, the amount of available N proved to be the limiting factor in the extent of decomposition of the Fucus by the bacteria. Since the decomposition of the humus in the mud results in the liberation of small amounts of N in an available form, the bacteria are thus enabled to bring about a considerably greater decomposition of the Fucus in the mud than in the water. This assumption was found to be correct, when, on adding small amounts of N in the form of ammonium salts or nitrates to the sea water, a great increase in the decomposition of the Fucus material took place. It is also possible that mud harbors certain bacteria which are not present in the water and which are able to decompose some of the specific carbohydrates present in the Fucus.

The nature of the organic residues in the sea may thus exert an important influence upon the amount of available N present at any one time in the sea water.

6555

Influence of Hyperpyrexia on Velocity of Blood Flow.*

MILTON KISSIN AND WILLIAM BIERMAN. (Introduced by E. H. Fishberg.)

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The radiotherm permits the study of hyperpyrexia uncomplicated by infectious disease. The body temperature can be raised or lowered at will. The radiotherm subjects the patient to the field of a high frequency radio oscillator and thereby induces hyperpyrexia (Hosmer,[1] Carpenter and Page[2]). The technique has been described fully by one of us (Bierman[3]).

The recent literature has been reviewed by Cheer,[4] Bazett,[5] and

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* This work was aided by a grant from the Herbert L. Celler Fellowship Foundation.

[1] Hosmer, Helen R., *Science*, 1928, **68**, 325.

[2] Carpenter, Chas. M., and Page, Albert B., *Science*, 1930, **71**, 450.

[3] Bierman, William, *Arch. Phys. Ther., X-Ray, Radium*, 1932, **13**, 383.

[4] Cheer, Sheo-Nan, *Am. J. Physiol.*, 1928, **84**, 587.

[5] Bazett, H. C., *J. Am. Med. Assn.*, 1921, **97**, 1271.

Wiggers and Orias.[6] Bazett summarized the differences and the similarities between fever and artificial hyperpyrexia. The pulse rate changes in the two conditions are similar and appear to depend on the temperature of the heart itself. Fever, especially during the rise of temperature, is associated with vasoconstriction in the skin, whereas vasodilatation occurs during artificial hyperthermia.

We studied the relation between body temperature as artificially elevated with the radiotherm and the circulation time. The method of determining the circulation time was devised by Robb and Weiss.[7] If one injects a small measured dose of sodium cyanide intravenously into an individual, a marked increase in the depth of respiration appears in 15 to 25 seconds. The latent period represents the time that it takes for the cyanide to go from the site of injection, the elbow, to the heart, through the right side of the heart, to the lungs, through the lungs, back to the heart, through the left side of the heart to the carotid artery. The time, from the end of the injection to the first deep breath, is measured with a stop watch. The interval is the circulation time.

Sodium cyanide, in proper dosage, is perhaps the most active of all respiratory stimulants (Loevenhart, Lorenz, Martin, and Malone[8]). It was formerly believed that the seat of its action was the respiratory center in the medulla (Loevenhart[9]), but Heymans, Bouckaert, and Dautrebande[10] showed that cyanide stimulates the respiration through the carotid sinus. Loevenhart calculated that sodium cyanide stimulates the respiration of the rabbit within a second after its appearance in the carotid artery.

Sodium cyanide for intravenous injection should be dissolved in physiological saline solution. It must be freshly prepared. It should not be sterilized lest the cyanide be decomposed. Cyanide is itself antiseptic, and the solution therefore will remain sterile. We used a 4% solution and injected 0.2 to 0.4 cc. according to body weight. A tuberculin syringe and a 20 gauge needle were employed. The duration of the injection was about a second. The end point was the time of the first deep breath but the patient always took several more after the first. It required 5 to 10 seconds for the respiration to return to normal. Sodium cyanide is destroyed very rapidly in the body, being transformed, presumably, into the non-toxic sulfocyanate.

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[6] Wiggers, Carl J., and Orias, Oscar, *Am. J. Physiol.*, 1932, **100**, 614.

[7] Robb, George, and Weiss, Soma, 1931, unpublished study.

[8] Loevenhart, A. S., Lorenz, W. F., Martin, H. G., and Malone, J. Y., *Arch. Int. Med.*, 1918, **21**, 109.

[9] Loevenhart, A. S., *Arch. Int. Med.*, 1915, **15**, 1059.

[10] Heymans, C., Bouckaert, Jean J., and Dautrebande, L., *Arch. Internat. de Pharm. et de Therapei*, 1931, **40**, 64.

The patients were those undergoing treatment for chronic disorders, arthritis, psoriasis, disseminated sclerosis, paresis, tabes, and mycosis fungoides. One patient had in addition a luetic aortic insufficiency. The circulation time was measured when the patient was placed between the plates of the radiotherm prior to turning on the current. The velocity was again determined in an hour or 2 when the temperature had reached 102 or 103°F. It was again estimated in 2 cases when the temperature had risen still higher, and in 3 instances it was measured after the patient had been removed from the radiotherm and the temperature had begun to fall.

TABLE I.

Diagnosis	Date	Hour	Mouth Temp.	Rectal Temp.	Pulse Rate	Circulation Time	Dosage of 4% NaCN
						Sec.	cc.
Gonorrheal arthritis	12-26-31	1:00 P. M.	98.6	99.2	62	20	0.2
		2:00 "	101.0	101.8	120	8	"
		3:00 "	102.6	103.6	108	6	"
Psoriasis	5-19-32	9:30 A. M.	97.0	98.2	66	23	"
		11:30 "	102.8	104.0	120	9	"
		2:50 P. M.	101.0	102.3	112	11	"
Multiple Sclerosis	6- 2-32	9:10 A. M.	98.0	98.0	64	23	"
		10:50 "	103.2	103.6	120	12½	"
		11:30 "	105.0	105.8	128	11	"
Tabo-paresis	6-19-31	9:20 "	97.4	98.0	80	26	0.3
Aortic Insufficiency		10:55 "	102.0	103.0	104	16	"
		11:40 "	104.4	105.8	126	6	"
Mycosis Fungoides	6-19-31	12:40 P. M.	99.4	100.2	100	11	0.4
		2:10 "	102.4	104.0	128	7	"
General Paresis	6-24-31	11:50 A. M.	98.7	99.6	80	17	0.25
		1:50 P. M.	102.0	103.6	108	10	"
		3:30 "	101.2	——	110	13	"

Results. The circulation time shortened as the body temperature rose, and conversely, the circulation time lengthened as the temperature fell. The blood velocity was increased during hyperpyrexia. The velocity was not always proportional to the temperature nor to the pulse rate but followed the temperature more closely than it did the pulse.

Other changes in the circulation, besides acceleration of the heart rate, play a rôle in the increased velocity of the blood that occurs during hyperthermia. These include the output of the heart per minute; the path taken by the cyanide; the peripheral resistance, and the viscosity of the blood.

Summary. The circulation time was measured with the cyanide method during hyperpyrexia induced by the radiotherm. Seventeen observations were made on 6 patients. In every case the circulation time diminished as the body temperature rose, being roughly proportional to the body temperature.

Conclusion. During hyperpyrexia, induced by radiothermy, there is an acceleration of the velocity of blood flow.

6556

Differences in Response of Female Macacus Monkey to Extracts of Anterior Pituitary and of Human Pregnancy Urine.*†

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Extracts either of the anterior pituitary or derivatives of human pregnancy urine cause follicular growth followed by rapid luteinization in the rodent's ovary. The present report emphasizes the difference in biological response between these 2 substances after a study of a considerable series of Macacus monkeys.

Ovaries of 31 experimental monkeys, ranging in body weight from 1800 to 5100 gm., have been studied. These animals were treated with anterior pituitary implants, with water soluble fraction of the pyridine extract of sheep anterior pituitary (Fevold, Hisaw and Leonard), with an extract of pregnancy urine (Zondek's method), and with Antuitrin S, also a pregnancy urine derivative. Animals were treated for 4 to 30 days, injections being made in most cases twice daily, subcutaneously or intravenously.

Hisaw and his associates[1] reported the effects of the pyridine extracts of the anterior lobe in causing follicular growth and changes in the sexual skin of monkeys. The action of pyridine extract on the ovary of the monkey in the present series (ovaries of 13 animals studied microscopically) may cause either the small cystic condition, similar to the effects obtained by implants (Allen, Hartman) or a rather uniform growth of all follicles depending on

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* Aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

† The dried sheep pituitaries and the Antuitrin S were supplied by Parke, Davis and Company, through the interest and courtesy of Dr. O. P. Kamm.

[1] Hisaw, Fevold and Leonard, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 204.

method of administration, state of the ovary at the time of treatment, total dosage and length of treatment. We have found, as Hisaw, *et al.*, had reported, that a prompt response of the sex skin occurs after this extract (reddening, turgor and a progressive pachydermatous folding characteristic of the monkey entering a normal adolescence). There are no failures in this external change when a potent follicle-stimulating extract is used.

In contrast to these findings is the complete absence of this type of response after treatment with derivatives of pregnancy urine. These extracts have all been found to have adequate potency when tested on immature rats. Ether-washed urine concentrate (1:60) equivalent to 2.6 liters of pooled whole pregnancy urine given first subcutaneously, later intravenously, for 32 days in a 2900 gm. animal (My 109) caused no change in the sex skin, no reddening nor any oedema or pachydermatous folding characteristic of the change following oestrin, implants or anterior lobe extract. Another animal (My 111) 3150 gm., received 8000 R. U. Antuitrin S (Rx095149A 200 RU/cc.) intravenously over a period of 22 days with no external change indicative of an increased follicular activity. *There is no change of the sex skin of any monkey with the gonadokinetic principles derived from human pregnancy urine,* regardless of total dosage, length of treatment, method of injection, or age or condition of the animal. Allen has shown that this change in the sex skin in an immature monkey can be induced with as little as 80 rat units of oestrin. There is therefore little or no oestrin produced as a result of this type of treatment.

Ovaries of 10 animals have been studied microscopically following treatment with such preparations. Follicular growth is not morphologically apparent. The medium sized and large follicles appear unchanged, although secretory activity may be checked. The small follicles with a small antrum are greatly changed into a variety of pseudocorpora atretica, by hyaline degeneration in which cell bodies are no longer determinable, but in which dense fibrous elements, distinctly hyalinized, often enclose a cytolized ovum. These structures may be seen in control ovaries of immature or adult animals, but are not numerous. The ovaries of animals treated with gland extract are increased in volume and weight from 4 to 10 times, while those treated with pregnancy urine derivatives remain uniform with the controls.

While the aqueous fraction of pyridine extract of the anterior pituitary activates the follicle, producing in 6 days the characteristic changes of the sex skin, 3 different preparations of pregnancy urine did not do so, even with large dosages and prolonged treatment. The only ovarian response is an increase in the number of corpora atretica of the small follicles only, and in the degree of hyalinization. The biological action of substances from these 2 sources is not the same when tested on the female Macacus monkey. These differences are more pronounced in the female than as seen in the male monkey (Engle), and add to the increasing mass of data that extracts of anterior pituitary and of human pregnancy urine are biologically different.

6557

Metabolic Differences Between Two Transmission Lines of Mouse Leukemia.*

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The experiments of Warburg[1] on the metabolic differences between tumors and normal tissues have stimulated similar investigations in leukemia. Although considerable data have accumulated relative to the metabolism of the cells in leukemia, many of the conclusions have been contradictory.

The following investigation has been undertaken as part of the program conducted in these laboratories in which the genetics, pathology and cytology of transmissible leukemia of mice have been reported by MacDowell, Richter and Potter.[2] The genetically controlled material developed in their studies is particularly suitable for metabolic experiments. The oxygen consumption and both aerobic and anaerobic glycolysis of normal lymph nodes and leuke-

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* This investigation was supported by a grant from the Carnegie Corporation, and an appropriation from the Research Fund of Columbia University.

[1] Warburg, O., *Biochem. Z.*, 1924, **152**, 51.

[2] Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1930, **51**, 659. MacDowell, E. C., and Richter, M. N., *J. Cancer Research*, 1930, **14**, 434. Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1930, **15**, 823. MacDowell, E. C., and Richter, M. N., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1012. MacDowell, E. C., and Richter, M. N., *Biol. Zentral.*, 1932, **52**, 266. Potter, J. S., and Richter, M. N., *Proc. Nat. Ac. Sc.*, 1932, **18**, 298. Potter, J. S., and Richter, M. N., *Arch. Path.*, 1933, in press.

mic lymph nodes of 2 distinct transmission lines, designated A and I, have been studied by one of us.

All animals were from strain C-58 in which 100% were susceptible to the transmitted disease. Line I killed the inoculated animals in 3 to 4 days, Line A in 6 to 8 days. Infiltration of the lymph nodes in Line I has been shown to be complete in 72 hours, while in Line A, 4 days.

Method. The animals were 6 to 8 weeks old. The experimental animals were of the same sex and almost invariably litter mates. Congested, hemorrhagic and necrotic glands were discarded. Four normal animals were sacrificed for each determination of the Q_{O_2}, $Q^{O_2}_{CO_2}$ and $Q^{N_2}_{CO_2}$ of the lymph nodes. In Line I, one or 2 animals were used; in Line A the lymph nodes of 3 mice were combined to obtain sufficient material. The quantity of tissue in each respirometer varied from 30 to 70 mg. moist weight. The nodes of Line I were used 3 days after inoculation, of Line A, 5 to 6 days after inoculation. The nodes were cut in strips about 2 to 3 mm. thick, while immersed in unbuffered or phosphate buffered Ringer's solution. The sliced tissue was placed in 1 to 2 cc. of solution in the respirometers, about 15 to 20 minutes after the animal was sacrificed.

Oxygen consumption and aerobic and anaerobic glycolysis were measured by the Warburg method[2] with the tissues in Ringer's solution in Fenn respirometers. The nodes were weighed at the end of the experiment after removal of excess fluid by blotting. All tissues were then fixed in Flemming's solution for cytological study. The moisture content of the tissues was estimated as 80% and the observed values were corrected by this factor.

Results. Table I is a summary of the experiments, showing for each series the range of metabolism, the mean, probable error and differences in metabolism, probable error of the difference and the ratio of the difference to the probable error. The differences are from 5.8 to 18 times the probable errors of the differences.

Not only are there striking differences between the metabolism of lymph nodes of normal and leukemic animals but also between those of the 2 different lines of transmissible leukemia. The O_2 consumption of nodes of Line A is greater than that of nodes of both Line I and normal. However, the aerobic glycolysis of Line I is almost 3 times as great as that of normal and Line A. Furthermore, the anaerobic glycolysis of lymph nodes of Line I is about 1.5 times as great as that of Line A and almost 3 times as great as that of normal.

TABLE I.

Metabolism of Lymph Nodes of Normal Mice and Those Inoculated with Transmissible Lymphatic Leukemias of Lines A and I.

	No. Determinations	Range	Q_{O_2} Mean	*Diff.* P.E.	Range	$Q^{O_2}_{CO_2}$ Mean	*Diff.* P.E.	Range	$Q^{N_2}_{CO_2}$ Mean	*Diff.* P.E.
Normal	10	6.3-10.6	8.37±0.31		1.7- 4.6	3.02±0.22		4.8-12.7	7.9 ±0.57	
Line I	10	8.0- 9.1	8.59±0.01		6.9-10.7	8.61±0.24		15.0-27.2	20.2 ±0.85	
Line A	9	9.5-11.9	10.48±0.17		1.5- 4.5	3.12±0.21		8.1-18.3	14.12±0.6	
Difference										
Line I—Normal			None			5.59±0.31	18		12.34±1.03	12.0
” A— ”			2.11±0.35	6.0		None			6.22±0.81	7.7
” I—Line A			—1.89±0.19	10.0		5.49±0.32	17		6.12±1.03	5.8

These differences observed in different lines of lymphatic leukemia offer an indication of the causes of discrepancies occurring in the literature. Further investigation of this problem is necessary to explain the fundamental factors involved.

6558

Effect of Products of Typhoid Bacilli upon the Tuberculin Reaction.

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In a study of the effect of bacterial products upon tuberculous animals, a filtrate* of a culture of typhoid bacilli, potent in material producing the *Shwartzman phenomenon,* was injected intravascularly into tuberculous guinea pigs which had previously received intracutaneous injections of bovine tuberculin.

Tuberculous guinea pigs were injected with 1-45 to 1-500 dilutions of bovine tuberculin. One or 2 days later 0.5 to 1 cc. of typhoid filtrate was introduced into the blood stream. At the time of the injection of typhoid filtrate, the skin at the sites of the tuberculin reactions was pink or bright red and edematous. One hour afterwards these areas became dull red. About 2-3 hours later a violet or purplish discoloration appeared, and within one day the skin had become obviously necrotic. The extent of necrosis was proportional to the severity of the tuberculin reaction, this relationship being clearly seen in guinea pigs simultaneously injected with a series of 3 different dilutions of tuberculin.

The following is the description of a typical experiment. A guinea pig weighing 450 gm., which had been infected 7 weeks previously, was injected intracutaneously with 0.1 cc. of 1:45 and 1:135 dilutions of old tuberculin. Two days later the reactions at the site of the injections were as follows:

I (1:45) Light redness and edema 10x10 mm. raised 2 mm.
II (1:135) Pink discoloration and edema 5x4 mm. raised 1 mm.
Immediately after the reading of the skin test 1 cc. of typhoid filtrate was injected intracardially. One hour after the injection the discoloration was dull red and 3 hours later the skin reactions were: I (1:45) Mottled dark purple area 7x7 mm., edema 10x10 mm., raised 2 mm. II (1:135) Dark purple area 4x3 mm., edema 5x4 mm., raised 1 mm. A few dark purple spots about 1x1 mm. were seen around both skin tests. One day later the purple areas described above appeared to be necrotic.

Purple discoloration was not observed in the control guinea pigs which had been infected and tested with tuberculin but had received no typhoid filtrate.

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* Received through the courtesy of Dr. Gregory Shwartzman.

In normal guinea pigs, the areas of skin injected with old tuberculin were not affected by a subsequent intravascular injection of a typhoid filtrate. Hence it is probable that the specific inflammation produced by tuberculin in the hypersensitive tissue, rather than products of the tubercle bacillus *per se,* prepared the skin for the action of the typhoid filtrate introduced into the blood stream.

Conclusion. The intravascular injection of a filtrate from the culture of typhoid bacilli produces hemorrhagic necrosis in tuberculous guinea pigs at the site of positive tuberculin reactions.

6559

Independent Variation of Biological Characters of Bacteria as a Result of Dissociation.

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Numerous observations on bacterial dissociation indicate that the changes occurring as a result of dissociation may involve many of the cultural characteristics. Although alterations in colonial morphology are the most obvious, many other characteristics may also undergo modification. De Kruif demonstrated changes in virulence[1] and acid agglutination optimum.[2] Profound serological alterations have been studied by Arkwright,[3] Schütze,[4] Savage and White,[5] Goyle.[6] The modifying effects of dissociation on many biochemical reactions, including fermentative capacities, have also been reported.[7] In a recent study of the fermentations of variants of *Salmonella schottmülleri,* Nungester and Jung[8] obtained R-variants which formed gas and others that did not; they observed that an S-culture may lose its capacity to form gas without change in colonial morphology. These observations, they concluded, were in accord with the concept of independent variation of bacterial properties.

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[1] De Kruif, P., *J. Exp. Med.,* 1922, **35**, 631.

[2] De Kruif, P., *J. Gen. Physiol.,* 1922, **4**, 387.

[3] Arkwright, J. A., *J. Path. and Bact.,* 1921, **24**, 36.

[4] Schütze, H., *J. Hygiene,* 1921, **20**, 330.

[5] Savage, W. G., and White, P. B., *Spec. Rep. Series, Med. Research Council,* London, 1923, **25**, 90.

[6] Goyle, A. N., *J. Path. and Bact.,* 1926, **29**, 149.

[7] Hadley, P., *J. Inf. Dis.,* 1927, **40**, 1.

[8] Nungester, W. J., and Jung, R. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 681.

Our studies on S- and R-variants of *Salmonella schottmülleri, Salmonella morgani* and *Shigella paradysenteriae (Flexner)* appear to demonstrate that many of the biological characters—serological, morphological and physiological—of these cultures are capable of independent variation during the dissociative and revertive processes.

Well-stabilized R-variants were obtained from the 3 normal S-cultures by growth in formalin (1:8000) beef infusion broth. Each of the R-cultures regularly produced 100% rough colonies on agar plates. The R-variants were then subjected to environmental conditions favoring reversion: (1) growth in 2% homologous immune serum broth; (2) growth in broth containing 2% vaccine prepared by heating an 18-hour growth of the original smooth culture for 4 hours at 56°C.; (3) rapid transfers in plain beef infusion broth. A variety of "reversion" cultures, derived from the 3 R-variants, was obtained. Serological, morphological and physiological characteristics of the 3 original S-cultures, the 3 R-variants, and the 11 "reversion" cultures were studied. Observations were made on colonial morphology, cellular morphology, motility, acid and gas production in carbohydrate media, formation of indol, saline stability, acid agglutination optimum, type of growth in broth, and antigenic composition determined by cross agglutinations and reciprocal absorptions. Alterations of antigenic behavior were such that it has been possible to demonstrate independent variation of the agglutinative, absorptive, and agglutinogenic properties of the antigens. A variant culture may show impaired agglutinative capacity with little or no alteration of absorptive and agglutinogenic properties. Other variants may show unimpaired agglutinative capacity and partial suppression of absorptive capacity, or the changes may affect the agglutinogenic capacity more than the other two. In brief, the agglutinative, agglutinogenic and absorptive properties vary independently of each other. Similarly, the other characters of these cultures varied independently of each other and of the antigenic characters. A motile S-variant of *Salmonella schottmülleri* formed acid from the same carbohydrates as the original culture, but on repeated tests failed to form any gas, and in serological behavior the only alteration was a decreased absorptive capacity. In this instance, therefore, gas formation and absorptive capacity had varied independently of the other characters. One R-variant of *Salmonella schottmülleri* was motile, another non-motile. An S-variant of *Shigella paradysenteriae* was unstable in 0.85% NaCl; another, indistinguishable in colonial morphology, formed homogeneous suspensions. A large number of such changes has been observed without evidence of linkage of any 2 characters.

There has been no uniform pattern for the altered characters of the R-variants, nor for those of the reversion cultures derived from them. We have found no evidence in studying these cultures that during either the dissociative or the revertive process the characters of a culture change hand-in-hand. The alterations occur in various groupings, and each character appears to be capable of varying independently of the others. In no instance did we observe complete reversion of all the characteristics. Only one of the 11 reversion cultures has reverted to complete antigenic identity with the original culture, but this culture showed for many weeks a difference in cellular morphology, growing in long filamentous forms.

6560

On the Mechanism of True and of Non-Specific Complement Fixation Reactions.

A. HAMBLETON. (Introduced by H. Wasteneys.)

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The complement fixation test for tuberculosis gives a non-specific or falsely positive reaction if a balanced physiological saline, akin to Tyrode's solution, replaces the plain 0.9% NaCl used in the test. The balanced saline which gives the strongest non-specific reaction contains 0.888% NaCl, 0.009% $CaCl_2$, and 0.003% $MgCl_2$; both Ca and Mg salts are required, and it is only over a very limited range of concentration of these salts that the non-specific reaction occurs. Petroff's[1] whole bacillus antigen is more effective than any other examined in producing this non-specific reaction, while "fat-free" tuberculo-antigens do not give this non-specific effect. In the absence of antigen, *i. e.*, in the anticomplementary control tubes, balanced saline assists hemolysis, so that one-third of the normal unit of complement may produce complete hemolysis.

The mechanism of this falsely positive reaction is as follows:

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* Aided by the National Research Council of Canada.

[1] Petroff, S. A., *Z. f. Tuberk*, 1923, **39**, 100; quoted by Willis, H. S., Laboratory Diagnosis and Experimental Methods in Tuberculosis, Springfield, 1928, 166.

1. The surface properties of the (Petroff) antigen particles are modified when incubated with complement and plain saline, presumably due to irreversible adsorption of protein from the diluted guinea pig serum used as complement; the isoelectric point of the washed antigen is changed from pH 3.2 to pH 3.7 by this treatment.

2. In the presence of balanced saline, the surface properties of the antigen undergo a further change, in that the particles agglutinate readily, and fix complement; *i. e.,* the antigen now acts as does an antigen sensitized by immune serum. The tendency of the antigen to agglutinate is a reversible one, which is present while the antigen is in balanced saline, and absent if the antigen is transferred to plain saline.

Further details of the factors involved in this reaction will be given elsewhere.[2] The critical Na to Ca ratio which will produce this non-specific reaction is also approximately the ratio of these ions present in the blood and in those balanced physiological salines which are essential to many forms of cell life; it is also the Na to Ca ratio, which, due to antagonism of the Na and Ca ions, exerts zero effect upon the stability of oil-water emulsions (Clowes[3]).

This non-specific reaction is an example of an immune reaction being duplicated by means of non-specific reagents, and the following experiments give some information upon the mechanism of complement fixation. A non-specific complement fixation reaction (complement + antigen + balanced saline), and a true positive reaction (immune serum + complement + antigen in plain saline), were carried out simultaneously, the same amount of antigen being used in each reaction. By preliminary titration, the amount of immune serum used in the latter reaction was so adjusted that the antigens in the 2 reactions possessed equal complement fixing power. Direct comparison of the 2 antigens then indicated that their particles had the same degree of cohesion, and when shaken with oil, they exhibited the same percentage partition between the oil and water phases. The only physical difference observed was in the electrical potential on the washed antigen particles.

Complement fixation thus appears to be produced by an antigen which has acquired appropriate surface properties, regardless of whether these properties were produced by immune or by non-specific reactions; *i. e.,* complement fixation in itself is not a specific immune reaction, but only a secondary effect due to the surface properties of suitably modified antigens.

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[2] Hambleton, A., *Can. J. Res.*, 1932, **7**, 583, 596.

[3] Clowes, G. H. A., *J. Phys. Chem.*, 1916, **20**, 407.

6561

Production in Dogs of a Syndrome Similar to Sprue by Diets Deficient in Vitamin B_2.

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The value of the vitamin B complex in the therapy of sprue in Porto Rico has been established.[1] In cases of severe anemia its oral administration effected reticulocyte rises and improvement of blood values. In other cases presenting glossitis and diarrhea without severe anemia, the symptoms were promptly and completely relieved. These observations suggested that lack of vitamin B might be an important factor in the etiology of sprue. Accordingly, experiments with 50 dogs have been carried out to ascertain the effect of prolonged feeding of diets partially but not completely deficient in vitamin B_2.

The animals used were as old and as large as could be obtained. They were kept under uniform conditions. The diet fed was a modification of the Goldberger "black tongue producing" diet. It was low in protein, high in fat and carbohydrate, and contained only the vitamin B present in rice polishings. It contained the following ingredients:

	gm.		gm.
White corn meal	4,000	Cotton seed oil	300
Ground California black-eyed peas	500	Cod liver oil	200
Washed casein	500	Salt mixture (Cowgill)	50
Sugar	300	Rice polishings	400

The corn meal, peas and casein were thoroughly mixed and cooked 2 hours in a steam cooker. The sugar, oil, rice polishings, and salt mixture were then added. This amount was given daily to 40 dogs. The animals were allowed as much of the diet as they would take.

Careful studies of the blood were made at regular intervals. A certain number of the dogs fed the diet deficient in vitamin B_2 acquired a condition similar in some respects to sprue in human beings. The remainder developed a syndrome to be described later. Stomatitis was present in a mild degree in all. The buccal mucous membrane was injected and contained a variable number of circumscribed yellowish aphthae. Glossitis was a marked feature. In the earliest stages atrophy of the papillae of the tip and sides of the tongue occurred, leaving a smooth, deep pink to red surface. As the condition progressed the color became a deeper red to a fiery scarlet. The atrophic process extended dorsally and medially until, in certain animals, only a few papillae remained at the base of the tongue.

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[1] Castle, W. B., and Rhoads, C. P., *Lancet*, 1932, **1**, 1198.

Gastro-intestinal disturbance was marked. At the height of the glossitis many animals partly or completely refused food of any kind. Vomiting was frequent and occasionally severe. Chronic diarrhea was a striking feature. The stools were sometimes watery but more often yellow, voluminous and semi-solid in consistence.

All of the animals lost weight and in many cases to such a degree that they became emaciated. The average loss in this series was 4.51 kilos or 24% of the weight at the beginning of the experiment.

The blood changes were definite in 60% of the animals and striking in 30%. The average red cell count of the latter group at the beginning of the experiment was 6.44 millions. At the low point of the anemia it was 3.105 millions. There was an average decrease of 3.337 millions or over 50%. The average hemoglobin value as done by the Sahli acid hematin method, using calibrated tubes and standards, was 87.7% at the outset and 56.4% at the low point. The average decrease of hemoglobin was 31.3%, or about 30% of the original value, less than the average percentage decrease in number of red cells. This corresponds with an average increase in color index of 0.263 and an average increase in mean corpuscular volume of 22.9 cubic microns. The sedimentation rate of the blood cells increased markedly at the height of the anemia.

Bone marrow studies revealed striking alterations in the animals with severe anemia. The marrow of the long bones of the dog is normally fatty and fairly acellular. At the height of the experimental anemia the fatty marrow was completely replaced by solid, deep purple-red tissue, macroscopically indistinguishable from the marrow of cases of pernicious anemia. Histological studies showed proliferation of endothelial cells and of megaloblasts with little tendency to maturation. The histopathological picture was quite similar to that seen in cases of sprue anemia or in pernicious anemia.

6562

Colorimetric Detection of Barbital and its Applications.

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Barbital and other barbituric acid derivatives are usually detected by recrystallization (Bachem[1]), and only few chemical tests for their identity have been described. Most of these tests are obviously crude and unsatisfactory, or at least not specific. One cannot test for the presence of barbital with Millon's reagent, Denigès' reagent, phenylhydrazine and sodium nitroprusside, or sulphuric acid and naphthol (Parri[2]). The murexide test of Handorf[3] is likewise not sufficiently specific. Parri,[2] Zwikker,[4] and Bodendorf[5] used cobaltous salts for the detection of barbiturates, but proved neither the specificity of their tests, nor did they attempt to make them sensitive beyond 1 part in 2000.

We devised the following test for the determination of barbiturates. We acidulate the unknown solution with hydrochloric acid, and shake with 10 volumes of chloroform in a separatory funnel. Six cc. of the chloroform extract are divided into 3 equal parts, A, B, and C. To A we add 0.05 cc. of a 1% solution of cobalt acetate in absolute methyl alcohol; to B, 0.1 cc.; and to C, 0.2 cc. of the same. This gives no color beyond a dilution of the original color of the cobalt acetate solution. Then to A we add 0.1 cc. of 1% barium hydroxide solution in absolute methyl alcohol; to B we add 0.2 cc., and to C 0.4 cc. of the same. If malonyl urea derivatives are present in the extract, a blue color always appears in A, may appear in B, or even in C, according to the amount of barbiturates contained therein. This step gives us an approximate idea of the concentration of barbiturates in the solution, for we have found that small amounts of barbital react only to small amounts of the reagents. If only A gives a blue color, the solution contains less than 0.25 mg. per cc.; if B also yields a blue color, the extract contains 0.5 mg. per cc. or less; if A, B, and C are blue, the extract contains 0.5 mg. per cc. or more.

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[1] Bachem, *Arch. Exp. Path. u. Pharm.*, 1910, **63**, 228.

[2] Parri, *Boll. Chim. Farm.*, 1924, **63**, 401.

[3] Handorf, *Z. f. ges. Exp. Med.*, 1922, **28**, 56.

[4] Zwikker, *Pharm. Weekblad*, 1931, **68**, 975.

[5] Bodendorf, *Arch. d. Pharm.*, 1932, **270**, 291.

The next step consists of matching the color produced by the unknown solution against the color produced by chloroform solutions of barbital of known strength in appropriate ranges. For example, if only A yields a blue color, we prepare 0.025%, 0.01%, and 0.005% solutions of barbital in chloroform and treat these samples in the same manner as sample A was tested, and match the color. This colorimetric test allows us a very accurate estimate of the barbital present in the extract. If we test in the ranges of samples B or C, that is, if B or both are positive, the procedure is the same, except that to the known strengths of barbital solution the same amounts of reagents are added as used in samples B or C respectively. For instance, if sample C also yields a blue color, we must match its color against that given by solutions of barbital in chloroform in concentrations of 0.5%, 0.2%, 0.1%, etc., again adding the same amounts of reagents as in C.

By means of this test we can detect barbital in chloroform extracts in concentrations of at least 1 in 20,000. This means that by concentrating the chloroform extracts we can actually discover the presence of barbital in body fluids in such small amounts as 0.05 mg. per cc. Care must be taken to exclude water in all procedures.

This test yields a blue color, *i. e.,* positive, only with barbital and other barbituric acid derivatives, but gives a negative, *i. e.,* pinkish, dirty yellow, or a green color, with every other compound. We have shown that both malonic acid and urea give negative tests, as do sulfonals, urethanes, uric acid, xanthine, adenine, cysteine, leucine, and allontoin. The sulphocyanates, and some other compounds, give a blue color with cobalt acetate which turns negative upon the addition of barium hydroxide. In short, this test is positive only with barbiturates.

Using this test, we attacked certain problems pertaining to the pharmacology of barbital. We found that barbital sodium following injection of doses of 300 mg. per kg. intravenously disappears from the blood stream in 6 minutes or less. After 3 minutes, only traces can be detected in the blood. We have also investigated the rate of excretion of barbital in the urine of normal dogs, and in dogs treated with diuretics.

Into each of 6 dogs we injected 375 mg. per kg. of sodium barbital intravenously. Three received no additional treatment. One of these dogs died in 12 hours, excreting 50 cc. of urine, which contained 500 mg. of barbital, or one-sixth of the total dose. The 2 remaining dogs recovered, excreting in 24 hours 1,230 mg., or 37%, and 2,100 mg., or 52%, respectively. In 48 hours they excreted

1,940 mg. and 3,060 mg., respectively, representing 58% and 76% of the total doses injected.

The other 3 dogs received, in addition to 375 mg. per kg. of sodium barbital by vein, a total dose of 2 cc. novasurol intramuscularly and 150 mg. per kg. of ammonium chloride subcutaneously. These injections produced profound diuresis in 2 dogs. The third died an hour after the administration; no diuresis had occurred, and 0.15% of the total injected dose was detected in the 23 cc. of urine produced. One dog lived 18 hours, in which time 7% of the total amount of barbital sodium injected was excreted. The last excreted 26% in 40 hours. In these experiments, diuretic measures contributed neither to the recovery of the animals nor to the excretion rate of barbital.

The study of the excretion of other malonyl ureas in the urine by this test and the application of the same in a clinical diagnosis of acute and chronic poisonings and in legal medicine will be reserved for the detailed communication.

The authors wish to express their thanks to Dr. John L. Gipprich, S. J., Regent of the Medical School, for his interest and criticism.

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Preparation of a Purified and Highly Potent Extract of Growth Hormone of Anterior Pituitary Lobe.

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Since the demonstration by Long and Evans[1] of the growth hormone of the anterior pituitary, various workers have confirmed the existence in alkaline extracts of the anterior lobes of animals of a growth-promoting substance. Van Dyke and Wallen-Lawrence[2] have suggested the use of totally hypophysectomized animals as a test object for determining the potency of extracts. The latter also made definite progress in the preparation of potent extracts which were less crude than those previously used.

We have been successful in preparing highly potent extracts of the anterior lobe of oxen. These extracts have been tested for

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[1] Long and Evans, *Anat. Rec.*, 1921, **21**, 62.

[2] Van Dyke and Wallen-Lawrence, *J. Pharmacol.*, 1930, **40**, 413.

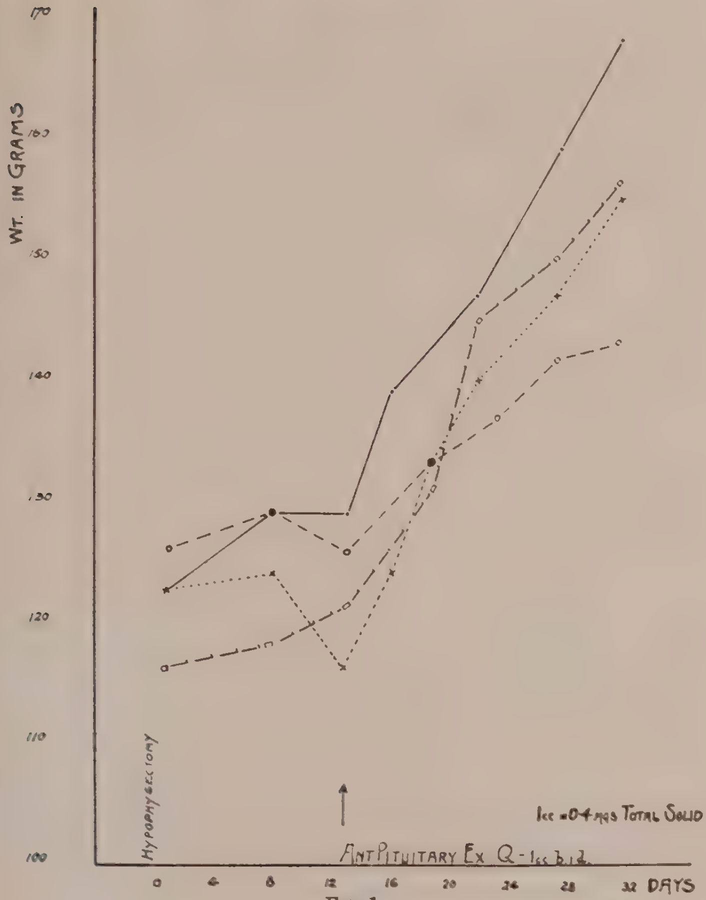

FIG. 1.

growth-promoting effects upon totally hypophysectomized rats. Growth has started almost immediately following the institution of therapy and has continued at a fairly uniform rate (see chart).

It was first observed that an alkaline extract (anterior lobes extracted with 10 volumes of 1% NH_4OH) which had been partially deproteinized, by the addition of acetic acid to pH 6.5 and filtering, had a slight growth-promoting effect in hypophysectomized rats. Such extracts were then saturated with ammonium sulphate in the presence of 1% NH_4OH, and the resultant precipitate collected, extracted with dilute ammonia and dialyzed. After dialysis the solution was concentrated at low temperature and pressure. During the concentration process a semi-crystalline substance separated out at a pH of 7.5 to 8. This was removed and an alkaline extract of it was found to be highly potent in stimulating growth in hypophysectomized rats.

From these leads the following method has been evolved. The anterior lobe tissue is treated with several volumes of dilute alkali: 0.5 to 1% NaOH or 1% NH_4OH. The mixture is acidified with acetic acid and filtered and the residue is again suspended in dilute alkali and reprecipitated with acetic acid and filtered. This extraction with dilute acetic acid as outlined may be repeated 5 times. Ammonia is added to the combined filtrates to give approximately a 1% concentration. By the addition of appropriate amounts of calcium chloride and sodium phosphate a suspension of $Ca_3(PO_4)_2$ is produced in the solution and the whole is concentrated at low temperature and pressure until the ammonia is practically all removed. The $Ca_3(PO_4)_2$ is then collected on a Buchner filter and is extracted repeatedly with 0.5% NaOH. The NaOH solution of adsorbed material which is thus removed is acidified with acetic to pH 6.5. It is then made alkaline with ammonia and concentrated at low temperature and pressure to remove the ammonia slowly. The semi-crystalline material which separates out at a pH 7.5 to 8 is removed and extracted with dilute sodium hydroxide. The alkaline solution is then almost neutralized and it may be used for assay at this point, 1 cc. representing approximately 2 gm. of original gland tissue. The total organic solids are between 1 and 2 mg. per gram of original anterior lobe tissue and ¼ cc. of such a solution administered twice daily to completely hypophysectomized rats has resulted in marked growth.

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Rough and Smooth Variants of Cl. Welchii.

ELIZABETH VERDER. (Introduced by Earl B. McKinley.)

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Rough and smooth variants of *Cl. welchii* have been described by several investigators.[1] All found similar colony types but report with no uniformity on the characteristics of the broth cultures of the variants. In the course of some work on the effect of varying gaseous mixtures and pressures on the growth of anaerobic organisms, the variants of 2* strains of *Cl. welchii* were studied.

Five distinct colony types were observed consistently on freshly poured blood agar plates containing about 5% horse or rabbit blood. The plates were incubated for 48 hours, either in desiccators in which a negative pressure of at least 28 inches of mercury was obtained with a cenco hyvac pump and maintained, or in desiccators which were alternately evacuated and refilled with nitrogen 3 times before sealing for the period of incubation. Little difference was noted in the characteristic growth of the variants when the cultures were incubated at 20°C. or 37°C.

The colonies of the 2 smoother variants differed in opacity and in their ability to spread but both gave glistening, homogeneous rounded colonies with entire edges. The thin transparent haloes of the spreading type were suggestive of those of the smooth motile colonies of some of the Salmonella strains. Rapid transfer in veal infusion broth (pH 7.2) of cultures, made from the smoother colonies when the strains were originally plated, resulted in the isolation of these 2 characteristically smooth variants. The broth cultures were incubated in an atmosphere of nitrogen in small anaerobic jars as suggested by Leifson.[2] The supernatant broth at first showed only slight clouding but gradually this increased until the broth was almost uniformly turbid; the slight sediment was of a very stringy, mucilaginous consistency. One strain gave a more mucoid growth than the other. Plates streaked from the turbid broth cultures gave at least 95% smooth colonies; the opaque non-spreading colonies were found in a higher percentage than the thin transparent ones. These colonies were solid regular masses of more or less lanceolate shape in deep agar.

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[1] Fortner, J., *Central. f. Bakteriol. Orig.*, 1929, **29**, 233; *Referat.*, 1930, **98**, 333. Condrea, P., *Compt. rend. Soc. de Biol.*, 1930, **103**, 631. Buchaly, J. F., *Central. f. Bakteriol.* 1931, **119**, 444.

* One strain was obtained from the Army Medical School, the other from the National Institute of Health.

[2] Leifson, *J. Bacteriol.*, 1931, **21**, 331.

The rough colonies were usually of a spreading type with finely fimbriated edges although many were deeply corrugated granular colonies with irregular, confined borders. The broth cultures of both showed a granular growth in the bottom of the tube with a clear supernatant broth. The colonies had a fuzzy appearance in deep agar. The only intermediate type gave a finely granular rounded colony that was deeply grooved in the center but sloped off to a smooth circular base; in broth cultures a granular sediment was covered with clear broth.

All of the colonies on blood agar were surrounded by clear zones of hemolysis. The morphology of the individual cells was quite varied; small coccoid forms and long filaments as well as the rods of usual size were found in many cultures. All strains produced spores in beef heart medium with a pH of 7.4 to 7.6.

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A Stereo X-Ray Method of Demonstrating Bronchoconstriction in Anaphylaxis and After Drugs.*

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Changes in bronchial tone in the anaphylactic guinea pig and in the unsensitized animal after the injection of bronchoconstrictor drugs have been successfully studied by 2 methods: (1) the perfusion of the isolated lung,[1, 2] and (2) the direct visualization of the bronchial tree by celloidin casts of the respiratory tract. The latter method, described by Hanzlik,[3] affords structural information of value not elicited by the physiologic technic and has the advantage of producing permanent casts for study and demonstration. The

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* Supported in part by the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

[1] Thornton, J. W., *Quar. J. Exp. Physiol.*, 1932, **21**, 305.

[2] Hurwitz, S. H., and Wessels, A. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 120.

[3] Hanzlik, P. J., *Am. J. Physiol.*, 1925, **72**, 558.

stereo X-ray technic presented in this paper has many of the advantages of this method, besides the additional one of greater simplicity and refinement and of giving a detail of finer structure of the bronchi, bronchioles, and pulmonary alveoli under normal and pathologic conditions not heretofore obtainable.

Method. The composition of the injection mass was a modification of that employed by Hill[4] for studies on the vascular system. For our work, we found a 12% bismuth oxychloride (Merck) suspended in a 20% solution of acacia the most satisfactory. This represents an approximate reversal of the percentages used by Hill, these being better suited for visualizing the respiratory tract. The acacia serves as an agent to aggregate the finely divided particles of bismuth, which fills the trachea, larger bronchi, bronchioles and alveoli and yet remains confined under the visceral covering of the lung.

The mixture was strained through cheese cloth into a wide-mouthed bottle into which was fitted and tightly fixed a rubber stopper with 2 holes. A long glass tube reaching to the bottom of the bottle was inserted into one hole and connected by a piece of rubber tubing to a short glass cannula for insertion into the trachea. A short tube fitted into the other hole was joined by a T-tube to a mercury manometer for gauging the pressure and to an outlet for air under pressure. The apparatus employed was the same as used by Hanzlik for celloidin injections, the details of which will be found in his paper.[3] A pressure of 60 mm. of mercury was found by trial adequate to fill the normal lung in from 5 to 10 minutes. The same pressure and time interval were employed for the pathologic lungs.

After introducing the injection mass, stereo X-ray films were taken of the lungs by the following technic: good exposures of guinea pig lungs were obtained by using a 1.5 kilowatt tube and 40 kv., 10 ma., 20 sec., and 38 inch. Excellent stereoscopic effects may be achieved by using a 6 inch shift, this being much wider than the usual one for such work.

Results. After completing an experiment on a guinea pig the lungs and heart were carefully removed *en masse* together with the trachea as far as the larynx and the bismuth-acacia suspension injected through a cannula into the trachea, the latter being ligated close to the end of the cannula after the injection is completed.

In typical bronchoconstriction of fatal anaphylactic shock in guinea pigs sensitized to horse serum only the trachea, larger bronchi and some small nests of atelectatic alveoli were filled. In 4 shocked guinea pigs showing mild symptoms or in animals which survive shock, varying degrees of filling of the bronchial bifurcations and lobes were observed. Six sensitized animals given a simultaneous intracardiac injection of a shock dose of serum in combination with epinephrine (0.2 mg. per kilo of body weight) survived. The lungs of these animals as well as those of 4 normal guinea pigs injected with serum alone or epinephrine alone showed complete filling.

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[4] Hill, E. C., *Johns Hopkins Hosp. Bull.*, 1929, **44**, 248.

The effects of other broncho-dilators on sensitized bronchi such as atropine in combination with serum are being studied.

Histamine in doses from 0.25 to 0.4 mg. per kilo of body weight produced varying degrees of bronchoconstriction. The injected lungs of 4 of these animals showed different degrees of filling of the bronchi, bronchioles and pulmonary alveoli. In some of the stereo X-rays actual beading of the smaller bronchi could be seen, points of constriction, alternating with points of relaxation, thus indicating that not all of the bronchioles are acted upon by the histamine.

These experimental observations suggested the possibility of demonstrating and analyzing calibre-changes in the bronchi of human subjects by stereo-roentgenography. We are accordingly employing lipiodal, introduced by tracheal catheter for visualization of the bronchi of allergic patients before and after the production of bronchospasm by the subcutaneous injection of antigen (pollen, horse dander), in carefully graded doses, and under controlled conditions. Results of these observations will be reported later.

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Evidence of Excretion of Iron Salts by Proximal Convolution of the Nephron of the Frog's Kidney.

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It is becoming increasingly evident that the glomerulus and tubule constitute functionally different sites for the elimination of the various urinary constituents. Several investigators have demonstrated that the proximal convolution of the vertebrate kidney is secretory in function. The studies of Holton and Bensley[1] on the mammalian kidney when it is excreting dyes and iron salts under conditions of very low blood pressure indicate a secretory process in the elimination of these substances. Verne,[2] however, studying the aglomerular fish kidney, shows that this type of kidney does not excrete iron salts in appreciable amounts. These studies suggested that the Nussbaum experiment, with modifications, might prove valuable in determining the possible function of the proximal convolution in the excretion of iron salts.

Male frogs *(Rana pipiens)* were used in the experiments, the account of which follows. The animals were anesthetized by injecting from 1.0 to 1.4 cc. of a 10% solution of urethane into the submaxillary lymph sac. Operation to expose the kidney was made by using a thermocautery. Pelvic lymph hearts were removed in order to eliminate possible lymphatic circulation, the coeliaco-mesenteric artery ligated, and the intestine removed. The peritoneum extending from the right kidney to the right body wall was cut through and the lateral branches of the renal portal were cauterized, permitting deflection of the kidney and exposure of the dorsal aorta and the urogenital arteries. The latter were pulled out to the right with a small hook, doubly ligated, and severed, care being taken to treat in like manner all branches of the aorta between the juncture of the coeliaco-mesenteric artery and the bifurcation of the aorta to form the iliac arteries. The right kidney was then replaced and both kidneys examined microscopically by means of reflected light. When the circulation in all visible glomeruli was completely stopped (usually from 10 to 15 minutes after completion of the operation), 1.0 cc. of a mixture consisting of 4 parts of a freshly prepared 1.0% solution of ferric ammonium citrate (green scale) and 3 parts of a 1.0% solution of potassium ferrocyanide was injected into the submaxillary lymph sac. This was immediately followed by injection of 0.5 cc. of a weak solution of india ink in order to establish a check on possible glomerular activity. The animal was then placed in an oxygen atmosphere.

After a period varying from 5 minutes to 4 hours the kidneys were excised, washed out with Ringer's solution followed by 5% trichloracetic acid, and placed in 5% trichloracetic for 18 hours.

Microscopic examination of sections cut 10 micra thick and stained in Mayer's acid carmine showed iron to be precipitated in the form of Prussian Blue as follows:

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[1] Holton, Sylvia G., and Bensley, R. R., *Am. J. Anat.*, 1931, **47**, 241.

[2] Verne, J., *Arch. d'Anat. Micros.*, 1921, **18**, 357.

1. In the peritubular capillaries.

2. In the proximal convolution: (a) From 5 to 40 minutes. No appreciable amount in either cells or lumen of the tubule. (b) From 40 minutes to one hour. Uniformly distributed in particulate form throughout the cells except an area at the periphery equal to one-third to one-fourth of the width of the epithelium. (c) From one hour 50 minutes to 4 hours. In the lumen of the proximal convolution in pale, blue-green, diffuse form.

3. In the distal convolution: (a) From 5 minutes to 2 hours and 20 minutes. No iron in either cells or lumen of the tubule. (b) From 2 hours and 20 minutes to approximately 3 hours. In finely particulate form in the lumen. (c) From approximately 3 hours to 4 hours. In larger aggregates of intense blue in the lumen, and in particulate form in the nuclei alone of some of the cells of the epithelium of the distal convolution.

India ink was present in glomerular tufts in one of a total of 42 experiments. This was taken to be evidence of incomplete ligation of urogenital arteries and the protocol was discarded.

To determine the site and extent of occurrence of iron in the tubule, as well as possible variations in amounts present, without recourse to serial reconstruction, several kidneys were macerated, the entire tubule isolated by teasing with glass needles and mounted for microscopic examination by the method of Edwards which was demonstrated to the author. This method has not as yet been published but the results obtained by its use are in part available.[3] Microscopic examination of these whole, mounted tubules confirmed all observations made of the sectioned kidney.

These results offer added support to the view of Holton and Bensley,[1] Stieglitz,[4] and Edwards[3] that excretion of iron salts is accomplished, at least in part, by the epithelium of the proximal convoluted tubule.

Grateful acknowledgement is made for the assistance of Doctors J. A. Dye, Cornell University, and J. Graham Edwards of the University of Buffalo.

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[3] Edwards, J. Graham, *Am. J. Phys.*, 1930, **95**, 493.

[4] Stieglitz, Edward J., *Am. J. Anat.*, 1921, **29**, 33.

6567

Sulfhydryl Compounds and Crystalline Urease.

JAMES B. SUMNER AND LLOYD O. POLAND.

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Perlzweig[1] states that both crystalline urease and extracts of jack bean meal give a positive sulfhydryl test with nitroprusside and that the more active preparations give stronger tests than the weaker ones. He estimates one of his samples of jack bean meal to contain the equivalent of 70 mg. of glutathione per 100 gm. and believes the substance in the meal to be glutathione, rather than cysteine, since the material is negative to the Sullivan reaction until after hydrolysis. He suggests that urease activity is due in part to the sulfhydryl compound and that it may be possible to separate a sulfhydryl activator from the urease proper.

We have independently observed that urease, prepared from jack bean meal by the Sumner procedure and twice recrystallized from 30% alcohol, produces a moderate amount of red color when treated with nitroprusside, ammonium sulfate and ammonia, and had ascribed this reaction to the presence of sulfhydryl groups attached to the urease molecule. It was recently shown that no co-enzyme for urease is present in the soy bean, or the jack bean.[2] However, in view of the statements of Perlzweig we have undertaken experiments to ascertain the connection between crystalline urease and the sulfhydryl groups.

Perlzweig refers to studies of Waldschmidt-Leitz and his students upon the significance of sulfhydryl compounds in enzymatic reactions.[3] Attention should be called to the fact that the claim made by Waldschmidt-Leitz that liver arginase is wholly inactive in the absence of sulfhydryl compounds, while it is active in the presence of hydrogen sulfide, cysteine, or reduced glutathione has been vigorously denied by Edlbacher, Kraus, and Walter,[4] as well as by Klein and Ziese,[5] who claim that arginase is never activated by cysteine or by glutathione, but instead is inhibited.

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[1] Perlzweig, W. A., *Science*, 1932, **76**, 435.

[2] Sumner, J. B., and Kirk, J. S., *Biochem. J.*, 1932, **26**, 551.

[3] Waldschmidt-Leitz, E., and collaborators, *Naturwis.*, 1929, **17**, 85; 1930, **18**, 644; 1931, **19**, 964; *Z. physiol. Chem.*, 1930, **188**, 17; 1931, **198**, 260.

[4] Edlbacher, S., Kraus, J., and Walter, G., *Z. physiol. Chem.*, 1932, **206**, 65.

[5] Klein, G., and Ziese, W., *Z. physiol. Chem.*, 1932, **211**, 23.

It is impossible to employ crystalline urease prepared by the Sumner procedure[6] directly for the nitroprusside test, since the preparation contains acetone, which itself gives an intense coloration with nitroprusside. Therefore we have prepared urease by the usual procedure, and have then recrystallized it from 30% alcohol. One sample of urease, after 2 recrystallizations, gave a nitroprusside test equivalent to about 0.02 mg. of glutathione per mg. of urease. On the other hand, the original jack bean meal, compared with pure glutathione, was found to contain per 100 gm., roughly the equivalent of 50 mg. of glutathione. Since this meal had 195 units of urease per gram, and since the urease had 135 units per mg., it may be calculated that of the total color-producing substance in 100 gm. of jack bean meal only about 3% could be credited to the urease. Furthermore, while the substance in jack bean meal which produces most of the color, is readily dialyzable through a collodion membrane, the substance in crystalline urease, responsible for the nitroprusside test, will not dialyse. We have dialysed from 1 to 3 cc. of urease, containing from 1600 to 4800 units, against 5 cc. of water for from 2 to 44 hours and have never been able to detect the least trace of sulfhydryl compound in the outer liquid, while we have found the dialysed urease to produce approximately the same amount of red color with nitroprusside as at the start.

Hence, it is certain that while jack bean meal contains a considerable amount of material which reacts similarly to glutathione, crystalline urease does not contain this substance, but does give a reaction with nitroprusside, either because the urease molecule contains sulfhydryl groups, or because the preparation contained some colloidal impurity which contains sulfhydryl groups.

Titration of urease with iodine, by a procedure similar to that used by Kühnau[7] for the determination of glutathione, shows that in dilute sulfuric acid 1 mg. of urease reacts with 0.1 cc. of N/500 iodine. Twice recrystallized and dialysed ovalbumin uses up only approximately one-half of this amount of iodine. The filtrate from urease, precipitated with trichloracetic acid, takes up no measurable amount of iodine. Urease was found to give a much weaker nitroprusside test after having been boiled than before, while, ovalbumin, as is well known, gives no nitroprusside test until after having been coagulated by boiling or by other means.

Having access to crystallization, it seemed rather absurd to attempt to separate the sulfhydryl compound from crystalline urease by adsorption on kaolin or any other adsorbent. Consequently we have recrystallized urease from 30% alcohol repeatedly in order to note whether it is possible to remove the sulfhydryl compound. After 3 recrystallizations the amount of color given by the nitroprusside test remains approximately the same as after one recrystallization. We believe, therefore, that the urease molecule itself contains sulfhydryl groups, or groups which give the nitroprusside test. These groups, if sulfhydryl, may account for the readiness with which urease is inactivated by silver, mercury and copper ions, and by quinone and other oxidizing agents, and why urease is protected by sulfhydryl compounds.

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[6] Sumner, J. B., *J. Biol. Chem.*, 1926, **49**, 435.

[7] Kühnau, J., *Biochem. Z.*, 1931, **230**, 353.

Finally, the dialysable substance in jack bean meal is not concerned with urease activity since urease which is freed from this material has high activity, and since addition of concentrated dialysate from jack bean meal exerts no favorable effect upon urease action.

We wish to thank Dr. D. B. Hand for his kindness in supplying us with pure glutathione.

6568

Transmission of Neurohumors in Animals by Other Means Than Blood and Lymph.

G. H. PARKER.

From the Zoological Laboratories, Harvard University.

It has been assumed that the contraction or expansion of dermal melanophores in fishes whereby these animals take on a light or a dark tint is dependent upon substances carried in the animal's blood and lymph (Parker[1]). Such an assumption appears to be justified by the fact that if a small amount of dissolved adrenalin is injected into the muscles of a dark chub, *Fundulus heteroclitus*, this fish within a quarter of an hour or less will become very light-colored. That the normal changes in tint shown by such a fish are brought about by other means than by dissolved substances carried in blood and lymph seems very probable from the following observations.

If a short transverse incision is made in the tail of a light-colored Fundulus, a dark stripe extending from the cut to the posterior edge of the tail will quickly appear. This stripe will gradually become lighter and lighter till in a few days it is no longer distinguishable from the rest of the tail. The transverse cut severs a number of radiating nerve fibers that pass out into the fish's tail and the dark stripe itself represents an area of denervation thus produced. That this stripe should be dark is due to the fact that the severance of the nerve fibers excites the discharge of an expanding neurohumor whereby the melanophores are made to enlarge and thus to darken the skin.

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[1] Parker, G. H., Humoral agents in nervous activity with special reference to chromatophores. Cambridge, England, 1932, 79 pp.

On placing a light-colored fish, whose stripe has become equally light-colored, on an illuminated dark background, the body of the fish will become fully dark in a little less than 2 hours. The stripe, however, will remain light-colored for some time, changing slowly to dark only in the course of a day to a day and a half. If now the completely dark-colored fish is put on a light background, the body of the fish will become fully light in from 4 to 5 hours, but the stripe will not lighten fully before somewhat over a day to over 2 days. Thus in both sets of changes the disappearance of the stripe is very slow as compared with the disappearance of the corresponding tint on the general surface of the fish. Thus in these changes the stripe lags well behind the general body.

The light coloration of the general surface of the fish is believed to be due to the discharge from a given set of nerve terminals of a neurohumor that induces a contraction of the melanophores and the dark coloration to the discharge from a second set of terminals of a neurohumor that will induce an expansion of the melanophores (Mills[2]). In the denervated stripe these neurohumors cannot of course be produced and the changes in such a stripe are believed to result from the transmission of the appropriate neurohumor from its region of production adjacent to the stripe into the stripe itself. This assumption is supported by the fact that when the stripes begin to disappear they start to fade first at their edges, after which the fading spreads to their interiors. Further it requires a stripe of 2 mm. width about twice as long to fade as one a single millimeter wide. For these reasons it is concluded that the fading of a stripe is due to an invasion by an appropriate neurohumor.

The rate of this invasion can be roughly measured and may be stated in averages to be for the dark-inducing neurohumor a millimeter in about 20 hours and for the light-inducing one a millimeter in about 26 hours. The periods of time implied in these rates are so long compared with the time it takes adrenalin to act, at most a quarter of an hour after injection, as to suggest at once an entirely different means of transmission in the two instances. It does not seem conceivable that the 2 neurohumors concerned with the color changes could be transmitted by blood and lymph whose flow, even at their slowest, would not be so retarded as is indicated by the rates given. Much more likely is it that the neurohumors are handed on from the adjacent innervated region into the denervated one through some other medium than blood and lymph and such a medium may well be the tissues themselves, whose cells next the nerve terminals of the innervated area may take up the neurohumor there produced and pass it on either through their cell sap or their outer lipoid coverings to other cells more nearly within the denervated stripe. Thus the neurohumor would make its way from the nerve terminals of the adjoining region to the denervated melanophores by steps that would of necessity be time-consuming and consequently consistent with the intervals observed as contrasted with what has been noted for transmission by blood and lymph. I, therefore, conclude that in Fundulus the neurohumors for the melanophores spread by tissue, probably cellular, means and hence with great slowness.

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[2] Mills, S. M., *J. Exp. Zool.*, 1932, **64**, 231, 245.

The explanation of neurohumoral spread just put forward is quite in accord with what must occur rather extensively in many of the lower animals as, for instance, in the coelenterates. In such animals as sea-anemones, coral polyps, hydroids, and the like the wall of the body, which may be relatively thick, is composed of 2 cellular layers, an inner entoderm and an outer ectoderm separated by a firm supporting lamella. Only the entoderm is next the digestive cavity and hence only its cells are in a position to absorb nourishment directly from the supply of digested food elaborated in that cavity. The ectoderm, on the other hand, a layer of great activity from the standpoint of both muscles and glands, can gain its nourishment only by having the digested materials passed on to it through the entoderm and the intervening supporting lamella. Such a transmission seems to be a necessary part in the economy of such creatures and must be accomplished by the tissues of these lowly animals in much the same way as has been suggested for the transmission of neurohumors in the tail of Fundulus. The coelenterates possess neither blood nor lymph and the nourishment of their parts not next the digestive cavity must of necessity depend upon tissue or cellular transmission. From an evolutionary standpoint this type of transmission unquestionably antedates that of blood and lymph and must be looked upon as the original method of transmitting materials, a method still of first importance among primitive types and retained to a significant extent even in the vertebrates.

6569

Effect of Dextrose-Alcohol Mixture upon Pulmonary Fat Embolism.

LOUIS G. HERRMANN. (Introduced by S. Bayne-Jones.)

From the Laboratory of Surgical Research, University of Cincinnati, College of Medicine, and the Chirurgische Universitäts Klinik, University of Zürich, Switzerland.

The therapy of post-traumatic pulmonary fat embolism has rereceived little attention. We have studied the effect of various organic and inorganic substances upon the fat globules which lodge in the capillaries of the lungs after the intravenous injection of a known quantity of sterile fat from an animal of the same species.

Twenty-four large Belgian hares and 4 dogs were used. Normal, healthy animals of the same litter were used in each series of experiments.

The sterile fat was warmed to 37° C., and injected into either the marginal vein of the ear of the hares, or into the saphenous vein of the dogs. The injections were carried out at the uniform speed of 1 cc. per minute and without undue force. Four animals were used in each series of experiments. Into each animal we injected 1 cc. of the warmed, sterile fat per kilo of body weight. One animal of each series was kept for the control. The other 3 animals were treated by an intravenous injection of 5 cc. per kilo of body weight of the following dextrose-alcohol mixture: Ethyl alcohol C. P. (96%) 3 parts; dextrose C. P. (25% solution in water) 7 parts.

Two of the remaining animals of each series received a second injection of the same quantity of this mixture at the end of 12 hours. Twenty-four hours after the injection of the fat, one of the last 2 animals was again treated by a third injection of the same quantity of the mixture intravenously. All of the animals of each series were killed by an intracardiac injection of sodium cyanide 48 hours after the fat was injected into the blood stream. Immediately after death the lungs of the animals were placed in a large quantity of 10% solution of formaldehyde in order to insure complete fixation.

The amount of intravascular fat in the lungs was estimated for each animal of the various series, by studying, under the high power of the microscope, numerous corresponding sections of the pulmonary tissue from all of the lobes of the lungs. These sections of the pulmonary tissue were cut with the freezing microtome and the fat droplets were stained by Sudan III. Methylene blue was used as the counterstain.

Those animals which received the intravenous injection of relatively large amounts of fat developed severe dyspnoea, cyanosis and extreme restlessness, but these manifestations of a disturbance in the aeration of the blood disappeared almost immediately after one intravenous injection of the dextrose-alcohol mixture in the proportion of 5 cc. per kilo of the body weight of the animal. It has also been shown that such a therapeutic effect can be produced consistently by a dose which is less than 25% of the lethal dose of the dextrose-alcohol mixture. The various doses of this mixture for the usual laboratory animals have been found to be as follows:

Minimum Effective Dose	2.5 cc. to 3.5 cc. per kg. body wt.
Therapeutic Dose	4.5 " " 6.5 " " " " "
Narcotic Dose	7.5 " " 10.0 " " " " "
Lethal Dose	20.0 " " 26.5 " " " " "

It was found that when a dose of 4.5 cc. to 6.5 cc. per kilo of body weight of this dextrose-alcohol mixture was given intravenously to healthy animals no untoward symptoms developed.

Results. The lungs of the animals which received 3 intravenous injections of the dextrose-alcohol mixture were almost free from intravascular fat, while all of the capillaries of the lungs of the control animals contained large amounts of fat. The amount of intravascular fat in the lungs of the other animals of the series appeared to be inversely proportional to the number of intravenous injections of the dextrose-alcohol mixture which they had received.

Volumetric determinations of the neutral fats, the fatty acids, cholesterol, and phosphatids in the venous and arterial blood, before and after the intravenous injection of the fat and before and after the treatment with the dextrose-alcohol mixture, are being made to determine the quantitative distribution of the fat in the various portions of the general and the pulmonary circulations and the rate of disappearance of the fat from the blood stream following this method of therapy.

Western New York Section.

University of Buffalo, December 17, 1932.

6570

Relation of Adrenal Cortex to Development of Scurvy.*

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It is well known that in scurvy (guinea pigs), marked changes develop in the adrenal. The cortex of this gland is rich in hexuronic[1] acid which has been shown by King to be vitamin C.[2] This vitamin, at least in the guinea pig, is not synthesized to any extent in the body and, therefore, its concentration in the adrenal cortex may be indicative of participation of the latter as an intermediary step in its utilization. If this is true, cortin may play some rôle in the process.

To test this hypothesis 3 series of guinea pigs with different amounts of cortin were used. The animals in each group (9 or 10 animals to a group) were balanced for sex and weight and placed on a pre-experimental diet of vitamin C-free ration and lettuce *ad libitum*, for one week previous to the experiment. At the beginning of the experimental period, the lettuce was discontinued. All animals not injected with extract were injected with one cc. of isotonic NaCl solution twice daily so that they would be subjected to the same manipulation.

Series I—Normal amount of cortin (intact adrenals). Group I—Basal diet. Group 2—Basal diet and 0.7 cc. of orange juice daily (one-half protective dose).

Series II—Added cortin (by injection). Group 3—Basal diet and 1 cc. of cortin twice daily (40 gm. whole gland per cc.).

Series III—One adrenal removed. Group 4—Basal diet. Group 5—Basal diet and 0.7 cc. orange juice.

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* Aided by a grant from the Carnegie Corporation of New York to the Carnegie Institution of Washington.

[1] Szent-Gyorgyi, A., *Biochem. J.*, 1928, **22**, 1387.

[2] King, C. G., and Waugh, W. A., *Science*, 1932, **75**, 357.

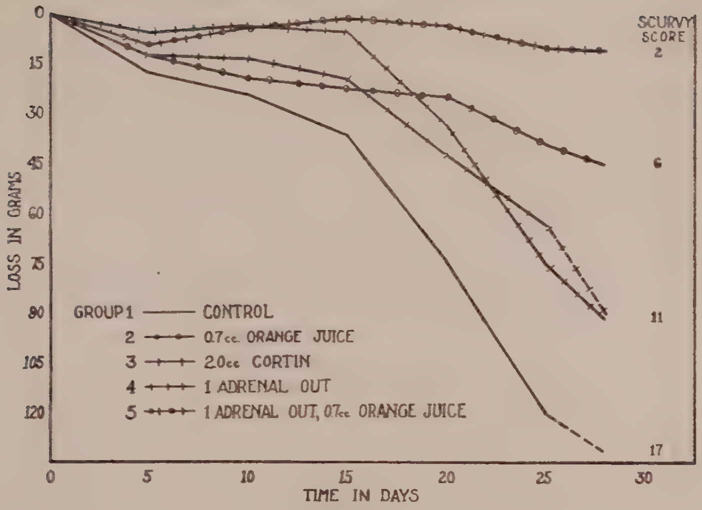

CHART 1.

The results are shown in the accompanying chart.

The guinea pigs on the control diet consistently lost weight from the beginning of the experiment which was carried out over a period of 28 days. This is not typical of a negative control series of vitamin C.[3] One or both of 2 factors may have been responsible, the amount of vitamin C obtained from lettuce in the pre-experimental period may not have been optimal or the frequent injections, in animals made sensitive by vitamin C deficiency, may have influenced the appetite. However, all conditions were controlled throughout the experiment so that the curves are comparable.

A plausible explanation for the results obtained with the cortin-injected animals is that cortin aids in the utilization of the vitamin C still available in the body. In animals with one adrenal removed, the curve resembles that of the cortin-injected guinea pigs. This may indicate a greater production of cortin than normal. It is known that the remaining adrenal after a single adrenalectomy hypertrophies[4] in young animals. It might be possible that there was overcompensation in the production of cortin for a time. (The adrenalectomy was performed at the beginning of the pre-experimental period.) This interpretation receives added support in the improved resistance to scurvy of Group 5 over Group 2.

The question may be raised whether our extract contains hexuronic acid. In preparation of the extract, ether was used for extraction. Hexuronic acid is insoluble in ether.

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[3] Sherman, H. C., and Smith, S. L., The Vitamins, 1931, p. 171.

[4] MacKay, E. M., and MacKay, L. L., *J. Exp. Med.*, 1926, **43**, 395.

These results may indicate that the adrenal cortex through cortin aids in the utilization of vitamin C.

6571

Effect of Cyanide, Hydrogen Sulphide and Carbon Monoxide on Response of Smooth Muscle to Drugs.

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Attention has been called to the fact that cyanide in low concentration may reversibly inhibit the stimulating action of drugs on the smooth muscle of rabbit uteri and intestinal strips.[1] The usual procedure* was to produce a contracture by the addition of a certain amount of drug to the solution bathing the muscle in the Trendelenberg apparatus. After washing out the drug, a solution of neutralized, buffered M/2000 NaCN was added. Three to 5 minutes later the drug was again added in the original concentration. This almost invariably failed to produce any contracture whatever. Washing and again testing the tissue with the same concentration of drug produced a contracture similar to that first obtained.

Garry[2] noted that the response of smooth muscle to certain drugs may be abolished by oxygen lack, and that the normal tonus of the strips was decreased by small amounts of cyanide. We find that cyanide in concentrations as low as M/200,000 is capable of exerting an inhibiting action on the response of the strips to drugs. This suggests the possibility that the effect may depend upon a heavy metal catalytic system such as Warburg has demonstrated to be involved in biological oxidation. To test this possibility we have studied the effect of hydrogen sulphide and of carbon monoxide on this system. Hydrogen sulphide in a concentration of M/2,000, completely blocked the action of acetyl choline (2×10^{-6}) on intestinal strips. Furthermore, it was found that mixtures of carbon monoxide and oxygen inhibited the action of adrenalin on uterus and of

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[1] Schmitt, F. O., and Nicoll, P. A., *Am. J. Physiol.*, 1932, **101**, 91.

* The expenses of this investigation were defrayed in part by a Research Grant to Washington University by the Rockefeller Foundation.

[2] Garry, R. C., *J. Physiol.*, 1928, **66**, 235.

acetyl choline on the intestine in the dark in concentrations in which a mixture of nitrogen and oxygen of similar percentage composition had no such effect. This indicates that carbon monoxide acts specifically in this case, the action being independent of asphyxial complications due merely to an insufficiency of oxygen. If such a carbon monoxide-poisoned preparation which has responded feebly to a drug be illuminated there is a fairly immediate increase in contracture which lasts throughout the period of illumination and which returns to the initial level upon discontinuance of illumination.

Some 25 experiments were performed on the blocking action of cyanide, 10 on the blocking action of carbon monoxide, and 4 on the light reversal of the carbon monoxide block. In none of these experiments have we observed any exceptions to the blocking actions of these substances or its reversal by light, as described above.

It would appear that some mechanism is involved in the response of smooth muscle to drugs and possibly also to autonomic nerve impulses which has the characteristics of a heavy metal catalytic system.

6572

Relative Depressant Effects of Certain Barbiturates on Heart of Elasmobranchs.

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Apparently no comparative study has been made of the relative cardiac depressant effects of the widely used barbiturates, perhaps because the effects on the central nervous system are so pronounced, as compared with the effects on the heart, that attention centers largely on their central actions.

Opportunity was afforded for studying the relative degree of cardiac depression produced by certain barbiturates, nembutal (sodium ethyl-methyl barbiturate), amytal (sodium iso-amyl barbiturate; luminal (sodium phenyl-ethyl barbiturate), and veronal (sodium di-ethyl barbiturate).

The study was carried out on the isolated heart of elasmobranch fishes, chiefly the *Squalus acanthias* or spiny dogfish, which is admirably adapted for such a study. The number of animals used totaled 14, all dogfish except one *Raia stabuliforis* (barndoor skate). The procedure for the dogfish heart was as follows:

The heart was removed from the animal with the sinus and great veins intact. A sino-auricular-ventricular preparation was prepared by cutting off the aortic arch and its vessels, then by means of a vertical incision the auricle on one side and about half the ventricle was cut off, thus exposing the endocardium. When the heart preparation was thus made, it was suspended in a balanced saline solution which was constantly supplied with oxygen and whose temperature was maintained at 16°C.

The balanced Locke-Ringer's solution was made by dissolving the following amounts of salts and urea in each liter of freshly distilled water (Barnstead still): Sodium chloride, 15 gm.; urea, 20 gm.; potassium chloride and calcium chloride, each 400 mg.; magnesium chloride, 1 gm.; sodium bicarbonate, 500 mg.; sodium acid phosphate, 100 mg.

When freshly distilled water was used, the pH of this solution was 7.55.

Graphic records obtained by means of a light heart lever were taken from the isolated rhythmically beating sino-auricular ventricular dogfish preparation and after a maximum rhythm was attained, 50 mg. of each of the above named barbiturates as the sodium salt was added to each 100 cc. of the saline solution bathing the heart preparation. This amount of barbiturate caused heart stoppage in a short time when nembutal, amytal and luminal were used, whereas when veronal was employed the heart rhythm persisted during the period of observation, which lasted for over an hour. While there was no great difference in the length of time required for this dosage of the 3 more toxic barbiturates to stop the heart, the degree of toxicity, judging by the average time required to produce heart stoppage, followed in a certain order.

Table I shows the individual variation for each series and the average time required. It also shows that these barbiturates fall into 2 groups, the more toxic being nembutal, amytal, and luminal; the least toxic being veronal, which does not stop the heart in this dosage after an hour or more.

As a further check on the relative toxicity between the least and one of the more toxic, namely veronal and nembutal, an experiment was carried out on the heart of the elasmobranch, *Raia stabuliforis* (barndoor skate). In this animal the heart is large enough to be divided into halves, and when mounted in separate chambers under identical conditions, the 2 halves will beat synchronously. Thus

TABLE I.

Barbiturate (as sodium salt)	Time to stop heart after 50 mg. Min.	Aver. Min.
Nembutal	4	7.5
	7	
	11	
	8	
Amytal	6	
	9	9—
	11	
Luminal	9	
	9	11—
	14	
Veronal	>68	
	>70	>68
	>157	

arranged, the usual dose (50 mg.) of each of the above was added to the saline. (Temp. 19°C.) and it was shown that nembutal stopped the heart preparation in 2 minutes, whereas veronal had little or no effect after several hours.

Conclusion. The depressant activity of nembutal, amytal, and luminal is high compared with veronal on the sino-auricular-ventricular preparation of *Squalus acanthias,* each given as the sodium salt.

6573

Maintenance Dose of Parenteral Liver Extract in Treatment of Pernicious Anemia.

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Gänsslen[1] and Castle and his co-workers[2, 3] have shown that the active principle of liver (fraction G of Cohn) is much more effective when given parenterally than when given orally to patients with pernicious anemia. Since this discovery, it has been of interest to know the average frequency of injections and the amount of active principle necessary to maintain a normal blood picture in a patient with pernicious anemia. Also is this quantity sufficient to maintain perfect health and a complete neurologic remission? Murphy[4] states that resistant cases seem to require extract derived from 100 gm. of liver at intervals of from 5 to 7 days while uncomplicated cases could probably be maintained with injections at from 10 to 20 day intervals. He observed improvement of neurologic symptoms. Connery and Goldwater[5] report 4 patients who received weekly injections of an extract derived from 100 gm. of liver over a period of from 4 to 5 months. The erythrocyte counts on many occasions were below 5,000,000 per cu. mm. No progress of neurologic symptoms was noted. Strauss and Castle[6] observed "satisfactory results" from injections at intervals of from 1 to 2 weeks over a period of 10 months. They state that there is little uniformity in the dose required by different patients. There was no progression of spinal cord symptoms and some patients showed continuous improvement.

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[1] Gänsslen, M., *Klin. Wchnschr.*, 1930, **9**, 2099.

[2] Castle, W. B., and Taylor, F. H. L., *J. Am. Med. Assn.*, 1931, **96**, 1198.

[3] Strauss, M. B., Taylor, F. H. L., and Castle, W. B., *J. Am. Med. Assn.*, 1931, **97**, 313.

The purpose of the observations here reported was to determine the minimum number of injections of extract derived from 100 gm. liver that would maintain an erythrocyte count at 5,000,000 per cu. mm. in patients with pernicious anemia. Results on 4 patients who received intramuscular injections of Lederle's concentrated solution of liver extract* (3 cc. derived from 100 gm. liver) over a period of 9 months are recorded in the chart. The grams hemoglobin per 100 cc. followed in general the curve of the erythrocyte counts and the color index was approximately unity in every case. Injections were given in the deltoid muscles of the 3 males and in the buttocks of the female patient. There was slight pain at the site of injection during and for a period of 8 to 24 hours following each injection, but there were no general reactions. During the 3 months prior to parenteral therapy, all the patients had some anemia which was probably due to inadequate oral therapy. No liver was taken by mouth after intramuscular therapy was instituted.

In the case of E. D. and W. G., the erythrocyte counts tended to fall below 5,000,000 when injections were given at 21-day intervals, whereas there was again an increase when injections were given at 14-day intervals. S. P., who received injections at 2 to 3

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[4] Murphy, W. P., *J. Am. Med. Assn.*, 1932, **98**, 1051.

[5] Connery, J. E., and Goldwater, L. J., *J. Am. Med. Assn.*, 1932, **98**, 1060.

[6] Strauss, M. B., and Castle, W. B., *J. Am. Med. Assn.*, 1932, **98**, 1620.

* The liver extract was supplied through the courtesy of Dr. G. W. Clark of the Lederle Laboratories.

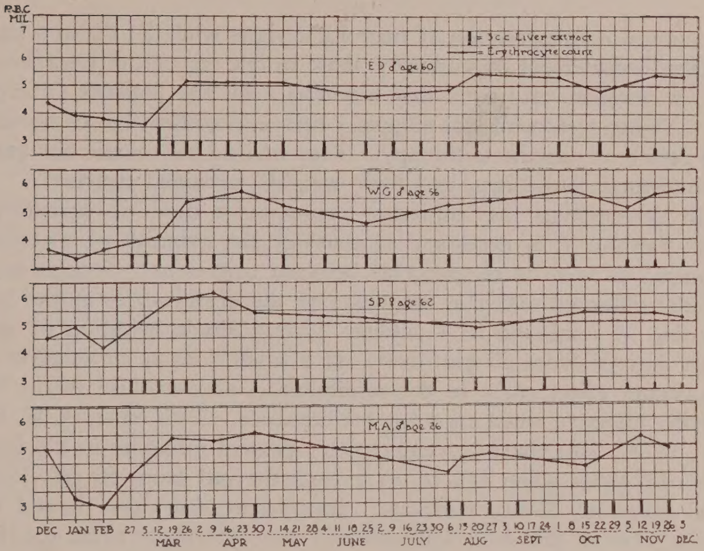

FIG. 1.

week intervals maintained an erythrocyte count at 5,000,000 per cu. mm. These 3 patients seem to have received about the minimum number of injections of extract derived from 100 gm. liver that would maintain a normal red blood cell count. The uniformity of the amount of liver given as shown in the table would undoubtedly not have been observed in a larger series of patients, especially when complicated.

TABLE I.

Period of Treatment	Liver from which extract derived	No. of injections	Ave. interval of injections
days	gm.		days
224	1400	14	16
224	1200	12	18.7
224	1200	12	18.7

Liver extract administered between March 26 and November 5, 1932.

In the case of M. A., when liver extract was stopped, the erythrocyte count gradually decreased. Later, injections at 14-day intervals were sufficient to raise the erythrocyte count to above 5,000,000 per cu. mm. The decrease noted on November 26th followed an acute respiratory infection.

At the beginning of intramuscular therapy, W. G. and S. P. showed evidence of slight cord involvement. After 9 months, W.

G. showed no progression of neurologic signs while S. P., who had marked impairment of vibratory sense in one leg before treatment, had a complete return of this function. All the patients (except M. A. when treatment was stopped) enjoyed excellent health during the period of parenteral therapy. They had no neurologic nor gastrointestinal complaints, and their weights remained constant.

In determining the minimum maintenance dose of parenteral liver therapy, it is necessary to consider the quantity of material required as well as the interval between injections. Strauss and Castle[6] observed maximal responses of the erythrocytes at the beginning of remission in 4 patients after a single injection of extract derived from 20 gm. liver. If a small dose at times gives a maximum effect early in the remission, a similar dose might be equally effective when used for maintenance. Two patients, E. D. and W. G. (see chart November 5th) showed increases in the erythrocyte count when 1½ cc. of extract derived from 50 gm. liver was given at 14-day intervals. Insufficient time has elapsed to know whether this dose will maintain the erythrocyte count equally as well as the larger dose.

In the routine treatment of pernicious anemia by parenteral therapy, it would seem advisable to give liver extract in excess of the minimum maintenance dose in order to allow a margin of safety in keeping the patient in a complete remission.

Summary. Four patients with pernicious anemia required extract derived from 100 gm. of liver at intervals of from 2 to 3 weeks to maintain a normal erythrocyte count.